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CORRECTION

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Page 122, para. below Table 1,

for: 'Liederkranz cheese containing the type B organism.'

read: 'Liederkranz Brand Pasteurized Process Cheese Spread containing the type B organism.'

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661. THE GENETIC CONNEXION BETWEEN BODY SIZE, MILK PRODUCTION AND EFFICIENCY IN DAIRY CATTLE

BY I. L. MASON,* ALAN ROBERTSON* AND B. GJELSTAD
Institute of Animal Genetics, Edinburgh

(With 2 Figures)

Many workers have calculated correlation coefficients between body size and milk production in dairy cattle, but some of these studies are confusing because of the manifold causes of variation which have been included. The animals concerned often differed in breed, age, stage of lactation or level of management, and the interpretations to be put upon the resulting figures therefore differ widely. In this paper we are interested in the main in the genetic variation within a single breed with a view to establishing the relative changes in the separate characteristics which would be brought about by selection.

The figures used all come from Red Danish cattle in Denmark, and are partly from records in commercial herds and partly from those in the special progeny testing stations. The analysis of those referring to production characteristics has already been published (9, 11), and full details will not be given here. The field records were the first complete annual records of 13,000 heifers in Jutland between 1946 and 1951, the majority being daughters of 152 Red Danish A.I. bulls. Milk yield and fat content were available for all animals and the heart girth (taken on the average about 2 months after calving) for about half the sample.

The progeny testing station records were taken from the annual reports for the years 1946–55 (1), and covered the progeny of 247 bulls (nine progeny groups being omitted because of disease and thirty-six because there was no other Red Danish bull tested at the same station in the same year). The body measurements used were the weight at reception at the station in September and the weight, heart girth and withers height in March (i.e. after milking about 5 months). The measurements were not taken simultaneously at all stations, although at any one station all animals were measured on the same day. The details of the procedure at the stations are already available in the literature (7, 8) and will not be repeated here.

RESULTS

(1) *Field records*

During the analysis of the milk records the herds were divided into three groups on the basis of the average yield. The relationship between girth and yield in the three groups is shown in Table 1. As the heifers in the three groups of herds are by the same bulls, the increase of mean girth with mean yield can be attributed to the dependence of both on level of feeding.

The variation in girth measurements within A.I. centres has been analysed for a sample of 139 bulls in twenty-eight A.I. centres with a minimum of five and an average of thirty-

* Member of the Scientific Staff of the Agricultural Research Council.

Table 1. *Relationship between herd average yield and girth of heifers*

	Mean yield (kg.)	Mean girth (cm.)	No. of heifers measured
High-yielding herds	4391	183·2	1528
Average-yielding herds	3917	181·8	1323
Low-yielding herds	3447	179·9	1271

Table 2. *Variance components for heart girth*

	cm.²
Within sires within herds (σ_w^2)	19·2
Between sires (σ_s^2)	2·1
Between herds (σ_h^2)	10·9

two daughters each. The method of analysis was the same as that used previously for milk yield (see table 5 in (9)). The within-herd within-bull component was estimated only on a sample of the data, comprising 593 degrees of freedom. The results are given in Table 2. In the analysis it was assumed that the sire-herd interaction was zero. From these figures, the heritability of girth measurements within herds can be calculated as

$$4\sigma_s^2/(\sigma_s^2+\sigma_w^2)=0{\cdot}40\pm0{\cdot}08.$$

Similar methods were used to analyse the covariance components between milk yield, fat content and heart girth. From these and the corresponding variances, within-bull and between-bull (genetic) correlations were calculated (Table 3).

Table 3. *Correlations between girth, milk yield and fat content*

	Milk yield	Fat content	Heart girth
		Within bulls	
Milk yield		−0·26	−0·06
Fat content	*−0·07*		0·06
Heart girth	*0·08*	*−0·17*	

Genetic correlations (between bulls)

The genetic correlations are calculated from the between-bull components of variance and covariance derived in the usual way from the analysis of variance. They differ from the simple correlations between the average figures for the separate bulls by the subtraction of the contributions of the sampling variance and covariance due to variation within groups. The within-bull figures are based on 593 D.F. and have therefore a standard error of 0·04 ($=1/\sqrt{593}$). The genetic correlations, based on 111 D.F. within A.I. centres, must have a greater standard error than the correlations between the bull averages with no correction for within-bull variation, i.e. the standard errors must be greater than 0·10.

The within-bull correlations need little comment. Only that between milk yield and fat content (−0·26) is significantly different from zero, and many similar figures are available in the literature. None of the genetic correlations differs significantly from zero. We can conclude that the genetic connexion between production characteristics and heart girth is slight.

(2) *Records from progeny testing stations*

In addition to measures of body size and production, we have also utilized records of the food consumed by the animals in the special stations to give us a measure of the over-all efficiency of food conversion. The measure used was the ratio of total fat-corrected milk to

food units consumed. This is equivalent to Dairy Merit or Gross Energetic Efficiency, as defined by Brody (3). As this is dependent on the feeding regime, the latter will be given in detail. Before calving (which occurs between September and November), the heifers are given roots, beet tops and concentrates to supply 7·06 Scandinavian feed units (1 FU ≡ 1 kg. barley) and 564 g. digestible true protein a day. After calving, the rations of roots, beet tops and hay are independent of milk yield. When in milk indoors, the heifers receive a basic ration of concentrates to supply 4·53 feed units and 400 g. protein and an additional 0·40 unit and 62 g. protein for each kg. of FCM. In the first 6 weeks, however, they are fed to a daily yield that is 2 kg. higher than their actual yield. It should be noted that the amount of food given is dependent on both milk yield and fat content, but is not dependent on body weight. At the end of the stall period in April they are turned out to grass and are fed supplementary concentrates depending on the condition of the grazing.

We had records from 247 progeny groups averaging eighteen heifers each. It has been shown by Johansson (7) and Venge (13) that there are significant differences in weight, yield and efficiency between stations and between years; all our analyses were therefore done within station-years—that is, for animals roughly comparable for age and calving date, milking in the same herd and all weighed and measured on the same day. The number of degrees of freedom between progeny groups within station-years was 150.

The variances and covariances of the various measurements were calculated within and between progeny groups. In an earlier paper (11) it was reported that the variance component between sires (σ_s^2) for milk yield was much greater in the stations than in the field, though the variance within groups (σ_w^2) was not. This led us to suggest that there were non-genetic causes of variation between progeny groups in the stations. For that reason, we feel cautious about ascribing the variance between sires in this analysis entirely to heredity. As the main source of statistical uncertainty was obviously the small number of degrees of freedom between sires, the components within progeny groups were estimated only from a restricted sample of thirty-six bulls evenly distributed in years and production level. The resulting estimates of the variance components are given in Table 4.

Table 4. *Variance components for measures of size and production*

	σ_w^2	σ_s^2	h^2 (apparent)	S.E.	Mean
Milk yield (10 kg. units)	6449	1072	0·57	±0·08	433
Fat content (%)	0·0642	0·017	0·84	±0·09	4·42
Efficiency (FCM/FU)	0·018	0·0025	0·48	±0·07	1·40
Weight at reception (kg.)	1889	220	0·41	±0·06	479
Weight in March (kg.)	1420	146	0·37	±0·06	504
Girth in March (cm.)	28	3·2	0·41	±0·06	184
Height in March (cm.)	9·5	1·4	0·51	±0·07	126

D.F. for σ_w^2: 641 for milk, fat, efficiency, and weight at reception; 516 for weight, girth and height in March.
D.F. for σ_s^2: 150 for all characters.

Comparable figures for some of the characters have been obtained before (11) from a section of the data. The apparent heritability of milk yield (0·57) is less than the 0·75 calculated by Robertson & Mason (11) from the results in the first 6 years. This decline is due to a fall in the between-sire component and a rise in that within sires in the last four tests. However, the value for the former component (1072 units) is still well above the value found in field records at the same level of production (412 units). The heritability of fat content remains high at 0·84. The results for March weight and gross efficiency are comparable

with those found by other workers (7, 13). The heritability of girth is in agreement with our figure from field records. As found by other authors (5, 10, 12) height appears to have a slightly higher heritability than girth.

However, there is one aspect of the girth figures which repays further examination. It will be noticed that the variance within progeny groups at the test stations (28 cm.^{2}) is greater than that within groups within herds in the field (19 cm.^{2}). The station records note the herd of origin of the heifers. It was found that if the variance within groups was also calculated within herds of origin it was reduced to 22 cm.^{2}. It seems then that, even in March, much of the original variation in girth between herds of origin still remains.

The correlations between the different characters based on the within-bull and between-bull components are given in Table 5. The latter are formally equivalent to genetic correlations.

Table 5. *Correlations based on between- and within-bull components*

	Milk yield	Fat content	Efficiency	March weight	Girth	Height
			Within bulls			
Milk yield		−0·30	0·84	−0·14	−0·16	0·07
Fat content	*−0·05*		−0·13	0·09	0·11	−0·08
Efficiency	*0·95*	*0·21*		−0·12	−0·16	0·04
March weight	*0·02*	*0·17*	*−0·03*		0·85	0·47
March girth	*0·02*	*−0·05*	*−0·02*	*0·74*		0·49
March height	*0·31*	*0·03*	*0·19*	*0·41*	*0·34*	
			Between bulls (apparent genetic correlations)			

There are 516 or 641 D.F. within bulls and 150 D.F. between bulls within station-years. This means that in estimating the significance of deviations from zero the standard error of the within-bull correlations can be taken as 0·04 (i.e. $1/\sqrt{n}$) and that of the correlations based on between-bull components will be somewhat greater than 0·08. An attempt was made to attach an empirical standard error to the latter correlations by dividing the data into five equal parts and observing the variance between the estimates from the separate parts. For correlations below 0·3 in magnitude the standard error appeared to be about 0·15, for those between 0·3 and 0·5 it was 0·10 and for the higher ranges it was 0·05.

Of the correlations within bulls, the interpretation of which is more certain, that between milk yield and fat content agrees with those calculated from field records and by other authors. The very high correlation between milk yield and gross efficiency may be surprising to some, but we shall see in the discussion that it is almost automatic. However, the fact that it is so does not make it any less important. The correlations between yield on the one hand and March weight and girth on the other, must be interpreted with caution. Gaines and his colleagues (4) noted that the correlation between body weight and lactation milk yield declines continuously as the weight is taken later and later in the lactation. The cows which come into milk in good condition tend to yield more as a result, and the heavy milkers tend to put on less flesh than others. This is strikingly illustrated by the correlations found in our material between lactation yield and body weight (Table 6).

Johansson (7) demonstrated a similar negative correlation between yield and increase in weight from calving to the end of the test. This was calculated on 743 cows in different years and stations. We have demonstrated that the same relationship applies to the

between-bull components, which have a preponderantly genetic origin. Withers height being a measure of skeletal size is less affected by these temporary influences and we find a positive, though not significant, correlation between height and yield.

Table 6. *Correlations between milk yield and body weight*

	D.F.	Initial weight	March weight	Increase in weight
Within bulls	516	0·19	−0·14	−0·42
Between bulls (apparent genetic)	150	0·25	0·02	−0·45

The negative correlation between fat content and efficiency is striking, considering that cows are fed according to fat yield (i.e. FCM). This seems to be a reflexion of the negative correlation between milk yield and fat content and the automatic connexion of the former with efficiency. Of the correlations between the measures of body size, that between girth and weight is the highest, as was found by Touchberry (12). This indicates that in this population body weight is determined more by variations in the fleshing of the animal than by variations in skeletal size.

It must be remembered that the variation between bulls is partly genetic and partly due to treatment common to members of a progeny group. In our comparative analyses of yield data from field and station records (11) we found the variance component between bulls considerably higher in the stations than in the field (standard deviations of 353 kg. within station-years and 203 kg. in the field). For this reason, we feel that a proportion of the variation between bulls in yield may not be genetic in origin and hence the correlations based on between-bull components must be interpreted with caution. The correlation between yield and efficiency is high, as it was within bulls. That between milk yield and fat content is less negative than that within bulls and correspondingly the correlation between fat content and efficiency is now positive. The other correlations are similar to those obtained within bulls.

DISCUSSION

A minor complication in these results is that the progeny groups differ significantly in age at calving (7), perhaps because of different periods of use of the sires concerned. Johansson showed that age at calving was significantly correlated with the average yield of the group and there is, of course, a correlation between age and weight. We therefore recalculated some of our correlations, keeping age at calving constant. The results shown in Table 7 are the justification for not bothering further with this complicating factor.

Table 7. *Correlations between size and yield based on between-bull components (apparent genetic correlations)*

	Over-all	Age at calving constant
Height and weight in March	0·41	0·36
Height and milk yield	0·31	0·26
March weight and milk yield	0·02	−0·07

The correlation between yield and gross efficiency is of considerable practical interest. Gross efficiency is here defined in terms of fat-corrected milk. The variation of FCM is in the main controlled by variation in milk yield because of the much greater coefficient of

variation of milk yield than of fat content. Adopting the simplifying assumption that the cow responds instantaneously to changes in intake, the process of reaching an equilibrium food level can be illustrated diagrammatically as shown in Fig. 1. The line *ABC*, given by the formula FU = 0·4 FCM + 4·6, represents the actual feeding standard (in which the milk yield determines the food input) and the two curves indicate hypothetical input-output relations for two cows (in which the food input now determines the milk yield). The input will reach stability at the points *A* and *B*, where the efficiency curve cuts the feeding standard line. Now at these equilibrium points the gross efficiencies are given by

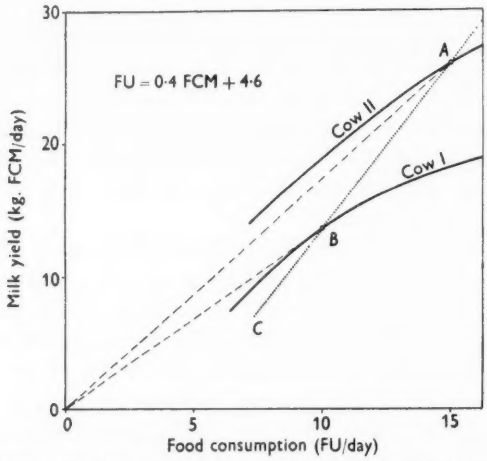

Fig. 1. The relationship between milk yield and feeding level (for explanation see text).

the slopes of the lines *OA* and *OB*. It follows mechanically that under a given feeding system which includes a basic ration, with or without a supplement, the highest yielding cow will also be the most efficient. The expected relationship between efficiency and yield is shown, for the Danish standards, in Fig. 2. The curve *AB*, given by the formula:

$$\text{Efficiency} = \frac{2{\cdot}5\ \text{FCM}}{\text{FCM} + 11{\cdot}5},$$

is calculated from the feeding standard and gives the same information as the line *ABC* of Fig. 1 in terms of efficiency instead of food intake. The dotted line represents the relationship which would be obtained if the regressions were assumed to be linear; its slope is 0·04 efficiency unit per kg. FCM per day.

To test the agreement of theory and practice we calculated the within-bull regression of efficiency on yield from a sample of our data (166 D.F.). It came to 0·00016 efficiency unit per kg. increase in lactation FCM. The total FCM yield is 216 times the highest daily yield (no figure is quoted for average daily yield). The observed regression thus represents $0{\cdot}00016 \times 216 = 0{\cdot}035$ efficiency unit per kg. FCM per day. This is close to the calculated figure of 0·04.

We can therefore say that, under a given system of feeding, selection for yield will automatically lead to an increase in gross efficiency in that system. In other words, as yield, and hence feed level, increases, the proportion of the feed which is attributable to

maintenance becomes less. The reasons for preferring any given system of rationing seem to be traditional rather than logical, as Blaxter (2) has pointed out.

The high correlation between yield and gross efficiency is reflected in the similarity of the correlations of the two with other characters. The important difference is that in the body-size measurements, the correlations between bulls are higher for yield than for efficiency. If we calculate the partial correlations between yield, efficiency and height we find $r_{EY.H} = 0{\cdot}91$, $r_{EH.Y} = -0{\cdot}33$. For a given milk yield, the taller animal is less efficient. But overall, the taller animal is more efficient because the increase in yield overweighs the

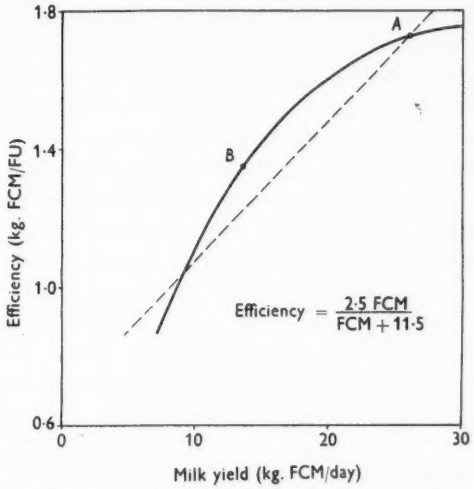

Fig. 2. The relationship between gross efficiency and milk yield.

increase in maintenance requirement. On this evidence, then, selection for yield or for efficiency would result in a slight increase in body size. In the absence of efficiency measurements, the best way would be to select on yield, making some allowance for height. In fact, it can be demonstrated mathematically from the above correlations that Y/H would correspond closely to the best index.

The relationship between body size and efficiency has been discussed also by Brody (3) and Wallace (14) though neither were primarily concerned with the genetic aspects. Brody concludes that 'dairy merit (i.e. gross efficiency) appears to be independent of body size as such'. He derives this from the observation that the relationship of milk energy yield to body weight is of the same form as that of maintenance requirement, i.e. both are proportional to $W^{0{\cdot}73}$. This approach seems to over-simplify the problem considerably, and furthermore ignores the energy used by low-producing cows in increasing their weight. It can be calculated from the data from the special stations that the variance between progeny groups in food used to increase body weight is about twice the variance in food required for maintenance. This would seem to indicate that dairy merit is mostly determined by the partition of the food between milk and growth during lactation.

Wallace (14) has considered the problem for cows whose requirements are entirely met by grazing, and in discussing efficiency he too ignores body-weight changes during lactation. He suggests that the best index of efficiency of the dairy cow is yield divided by the

square of the girth, whereas we have suggested yield divided by height. However, both these indices will have a very high correlation with yield itself because of the much higher coefficient of variation of the former than of body measurements. But selection on one of these indexes will reduce the correlated response of body size that selection on yield alone would cause.

The initial reason for this investigation was the light it might throw on the inter-relationships between body size and production. An important point that has arisen is that, for a useful discussion of the problem, we have to be more precise about what we mean by body size. We used three measurements, withers height, chest girth and body weight. The first is distinct from the other two in that, as a skeletal characteristic, it is not affected by temporary changes in response to stress—in particular in dairy cattle to the stress of lactation. The correlation of body weight with lactation yield decreases markedly during the lactation. We had measurements of girth both from the field and from the stations, the former being taken at about the second month of lactation and the latter after approximately 5 months. The within-bull correlations with yield were in both cases slightly negative ($-0{\cdot}06$ and $-0{\cdot}16$). The correlations based on between-bull components were, on the other hand, slightly positive ($0{\cdot}08$ and $0{\cdot}02$), though neither figure approached significance. In the stations, body weight behaved in the same manner as girth, with which it showed a high correlation. Both in the field and in the stations, girth had a positive within-bull correlation with fat content ($0{\cdot}06$ and $0{\cdot}11$) which became negative between bulls ($-0{\cdot}17$ and $-0{\cdot}05$). The former may be a reflexion of the effect of condition on fat content, which has been shown in experiments on the effect of plane of rearing on production (6). The latter suggest a small negative correlation between fleshing and fat content of the milk.

Height at withers (a measure of skeletal size) shows a positive correlation with milk yield both within and between bulls, both bordering on the 5% significance level. It would seem, therefore, that there is probably a positive genetic connexion between skeletal size and production.

On this evidence, selection for milk yield alone would produce a taller cow with rather less fleshing and a tendency to convert flesh into milk during lactation. But the magnitude of all of these correlations between yield and body measurements is small, and determined selectors could easily prevent the correlated response and even reverse its trend, at the expense of some decrease in the response of the primary character.

Finally, we should add a note of caution. These conclusions as to the inter-relationships between the various characters refer to one breed only and, what is more important, to one particular set of treatments and feeding standards. Their applicability to other feeding regimes, and in particular to conditions in which no concentrates at all are fed, is uncertain.

SUMMARY

1. An analysis has been made of production records and body measurements of dairy cattle from farms and special progeny testing stations in Denmark.

2. Heritability estimates of various measurements of body size and also of gross efficiency of food conversion were all in the neighbourhood of 0·50. Body weight was more closely correlated with girth than with height.

3. Milk yield had a very high correlation, both phenotypic and genetic, with gross

efficiency. This was shown on theoretical grounds to be a necessary result of any standard system of feeding.

4. Correlations between production characteristics and body measurements were slight. There was some suggestion that selection for yield would increase skeletal size but decrease fleshing.

5. The best index on which to select for efficiency of food conversion under these conditions would be the yield divided by the height.

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662. THE COMPARATIVE EFFICIENCY OF SOME METHODS OF ESTIMATING THE LIVE WEIGHT OF DAIRY COWS

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A knowledge of the live weight of individual cows has useful applications in the allocation of rations, the estimation of productive efficiency, and in computing sale value at the end of productive life. Weighing machines suitable for mature cattle are rarely installed on commercial farms, and, consequently, many studies of methods of estimating live weights from body measurements have been made in both America (1,2,3,4,5,6,7) and Europe (8,9,10,11,12). It is generally accepted that chest girth is the measurement most closely related to live weight and general body size (7,11,13), and its use appears to be justified by its high repeatability (13,14), ease of measurement and its relationship with both skeletal size and the overlying flesh cover.

The problem of estimating weight has been approached in several ways. Over a considerable range of live weight the relationship is best expressed (7) by the formula

$$Y = A(X)^b,$$

where Y = live weight, X = chest girth and A and b are constants. This is merely one application of the allometric relationships between the size of parts of the animal body and the size of the whole body discussed by Huxley (15), and the formula can be readily converted to a linear form for analytical purposes by taking logarithms, when

$$\log Y = \log A + b \log X.$$

This relationship has been used in some of the American work (3,4,6,7), and most of the remainder of the work already cited was based on the direct regression of live weight upon chest girth in groups of cattle of similar age and condition, since, in animals of similar chest girth, weight increases with condition and age (4,11).

In addition to these relatively recent studies of the direct relationship between chest girth and weight, a number of methods were recommended during the nineteenth century (8,16,17) which involved the computation of indices of body volume. This was usually done either by regarding the body as a cylinder, when

$$\text{volume} = K\ [\text{length of body} \times (\text{chest girth})^2]$$

or as the frustrum of a cone when

$$\text{volume} = K\ \{\text{length of body}\ [(\text{chest girth})^2 + (\text{paunch girth})^2 + (\text{chest girth} \times \text{paunch girth})]\}.$$

The objects of the present study were to describe the relationships between weight and body measurements in Dairy Shorthorn cows, and to study the effects of taking additional measurements on the accuracy of prediction of the logarithmic and body volume index methods, since practically no comparative studies have previously been reported.

MATERIAL AND METHODS

The data were collected from three Dairy Shorthorn herds during 1949–52, as part of a study of seasonal effects upon productivity under different conditions of herd management (18,19). These herds (19) and the methods of recording body measurements (13) have been described elsewhere. The following measurements were used: chest girth (*CG*), paunch girth (*PG*), length of body (withers to pins) (*LB*), depth of chest (*DC*) and width at hooks (*WH*). Live weights were recorded on one day only, immediately after the morning milking. Observations were made at 3-monthly intervals for 2 years, and consequently many of the cows studied provided more than one set of measurements. Data from pregnant cows measured more than 20 weeks after service were omitted because of the marked influence of the uterine contents on live weight at this stage (20,21), and a single live weight and its corresponding measurements were randomly selected for analysis from the remaining data for each cow. A total of 133 sets of measurements were available from animals which had calved at least once before measurement.

Multiple regression analysis was carried out, using the usual methods (22,23).

RESULTS

Differences between herds. The mean values of the live weights, body measurements and the ages of the cows in each herd are shown in Table 1. Analysis of variance showed that there were significant between-herd differences in live weight and body measurements but not in age. The mean values of live weight and body measurements were markedly lower in herd C than in the other two herds, and this can be ascribed to the considerable differences in herd environment which have been described elsewhere (19), and which were shown to have adverse effects upon productivity in herd C.

Table 1. *Means of weights and body measurements by herds*

Herd	No. of cows	Mean live weight (lb.)	Mean chest girth (*CG*) (in.)	Mean paunch girth (*PG*) (in.)	Mean length of body (*LB*) (in.)	Mean width at hooks (*WH*) (in.)	Mean depth of chest (*DC*) (in.)	Mean age (weeks)
A	27	1131	75·2	90·4	55·3	21·8	28·2	283
C	66	980	70·5	84·9	54·6	20·9	27·1	337
D	40	1193	76·5	90·4	56·9	22·6	28·9	327

The data from all three herds were pooled for subsequent analysis, since preliminary work showed that within herd regressions of log live weight on log CG gave no significant reduction in residual variance compared with that associated with the over-all regression, and since the object was to describe relationships between live-weight and body measurements applicable to Dairy Shorthorn cows over a wide range of age and condition.

Logarithmic methods of estimation. The regressions describing the relationships between the logarithms of single body measurements and the logarithm of live weight are shown in Table 2. The best estimate of log live weight was obtained from its regression on log *CG* which accounted for 90% of the original variance in the former. The residual variance from regressions with the remaining measurements was much higher and ranged from 18% for log *PG* to 32% for log *LB*.

The effect of adding each of the other measurements singly to the regression of log live

weight on log *CG* is shown in Table 3. In all cases the reduction in residual variance obtained was statistically significant, but the reduction was not very great with log *WH* or log *DC*. The addition of log *LB* or log *PG* reduced the residual s.s. by 30%.

Table 2. *Regressions of the logarithm of live weight* (*Y*) (*lb.*) *upon the logarithms of body measurements* (*X*) (*in.*)

Independent variate	Regression equation	Standard error of b	Residual s.s. for Y	Residual s.s. as % of total s.s. for Y
—	—	—	0·562722	100
Log *CG*	$Y = 2{\cdot}390X - 1{\cdot}429$	±0·0678	0·053735	10
Log *PG*	$Y = 2{\cdot}147X - 1{\cdot}143$	±0·0924	0·099773	18
Log *DC*	$Y = 2{\cdot}674X - 0{\cdot}836$	±0·0966	0·108921	19
Log *WH*	$Y = 2{\cdot}148X - 0{\cdot}163$	±0·1071	0·133876	24
Log *LB*	$Y = 2{\cdot}834X - 1{\cdot}915$	±0·1246	0·181146	32

Table 3. *Multiple regressions of log live weight* (*Y*) *upon log CG*(X_1) *and a second measurement* (X_2)

Second independent variate	Regression equation	Residual s.s. in Y	Residual s.s. as % of residual s.s. after regression on X_1 only
—	$Y = 2{\cdot}390X_1 - 1{\cdot}429$	0·053735	—
Log *PG*	$Y = 1{\cdot}659X_1 + 0{\cdot}796X_2 - 1{\cdot}612$	0·037611	70
Log *LB*	$Y = 1{\cdot}913X_1 + 0{\cdot}873X_2 - 2{\cdot}061$	0·037802	70
Log *WH*	$Y = 1{\cdot}915X_1 + 0{\cdot}543X_2 - 1{\cdot}268$	0·046403	86
Log *DC*	$Y = 1{\cdot}953X_1 + 0{\cdot}569X_2 - 1{\cdot}436$	0·050184	93

Table 4. *Progressive analysis of variance of log live weight*

Independent variates	D.F. Regression	D.F. Residual	s.s. due to Residual	s.s. due to Regression	s.s. due to Last variate	F (last variate)
—	0	132	0·5627224	—	—	—
Log *CG*	1	131	0·0537352	0·5089872	0·5089872	1·241**
Log *CG*, log *PG*	2	130	0·0376103	0·5251121	0·0161249	55·73**
Log *CG*, log *PG*, log *LB*	3	129	0·0270947	0·5356277	0·0105156	50·07**
Log *CG*, log *PG*, log *LB*, log *DC*	4	128	0·0270349	0·5356875	0·0000598	0·065 (N.S.
Log *CG*, log *PG*, log *LB*, log *DC*, log *WH*	5	127	0·0269641	0·535758	0·0000705	0·033 (N.S.

$$Y = 1{\cdot}3858X_1 + 0{\cdot}6628X_2 + 0{\cdot}7246X_3 - 2{\cdot}1075,$$
$$t = 13{\cdot}3^{**} \quad 7{\cdot}14^{**} \quad 7{\cdot}07^{**},$$

where Y = log live weight (lb.), X_1 = log chest girth (in.), X_2 = log paunch girth (in.), X_3 = log length of body (in.).
Statistical significance is indicated thus: ** Highly significant ($P > 0{\cdot}01$); N.S. Not significant.

The remaining variates (log *LB*, log *DC* and log *WH*) were added successively to the regression of log live weight on log *CG* and log *PG* with the results shown in Table 4. The inclusion of log *LB* produced a significant reduction in residual variance, but the effects of the subsequent addition of log *DC* and log *WH* were not appreciable or significant. The regression equation of log live weight upon log *CG*, log *PG* and log *LB* is shown at the bottom of Table 4, together with the values of '*t*' for the respective regression coefficients, which were highly significant.

There were some peculiarities when log age (weeks) was included in the analysis. When this variate was added to regressions including log *CG*, or log *CG* and log *PG* only, there was no significant reduction in residual variance, but when added to regressions involving log *LB* (i.e. log *CG* and log *LB* or log *CG*, log *PG* and log *LB*) its contribution

was significant. The regression equation of log live weight (Y) upon log CG (X_1), log PG (X_2), log LB (X_3) and log age (X_4) was

$$Y = 1{\cdot}296X_1 + 0{\cdot}700X_2 + 0{\cdot}993X_3 - 0{\cdot}041X_4 - 2{\cdot}378,$$

and the regression coefficients were all highly significant.

The residual variation in log live weight after the use of various regressions is shown in Table 5. The inclusion of log PG, in addition to log CG, reduced residual variance by 30%, the further inclusion of log LB by a further 20%, and of age by a further 8%.

Table 5. *Residual variation in log live weight* (Y) *after different regressions*

Independent variates	Residual s.s.	Residual
Log CG	0·053735	100
Log CG + log PG	0·037611	70
Log CG, PG, LB	0·0270947	50
Log CG, PG, LB and age	0·0223049	42

Methods using indices of body volume. The regressions of live weight (lb.) upon

$$\frac{LB(CG)^2}{1000} \text{ (index I)} \quad \text{and upon} \quad \frac{LB(CG^2+PG^2+CG.PG)}{10{,}000} \text{ (index II)}$$

are shown in Table 6. Both these regressions accounted for over 90% of the original variance of live weight, and the former was slightly the more effective.

Table 6. *Regressions of live weight* (Y) *on body volume indices*

Independent variate	b	Regression equation	Residual s.s.	Residual as % of total s.s.
—	—	—	3,358,901	100
(1) $\frac{LB \times CG^2}{1000}$ (index I)	3·345±0·0782	$Y = 3{\cdot}345X + 72{\cdot}1$	224,627	5·5
(2) $\frac{LB(CG^2+PG^2+PG.CG)}{10{,}0000}$ (index II)	9·351±0·1975	$Y = 9{\cdot}351X + 56{\cdot}9$	185,492	6·7

Table 7. *The effect of the addition of age upon the regression of live weight* (Y) *upon indices of body volume*

Independent variates	Regression equation	Residual s.s.	Reduction in residual s.s. due to age: Actual	Reduction in residual s.s. due to age: Percentage
$\frac{LB \times CG^2}{1000}$ (index I) (X_1) and age (weeks) (X_2)	$Y = 3{\cdot}462X_1 - 0{\cdot}105X_2 + 71{\cdot}1$	197,502	27,125	21
$\frac{LB(CG^2+PG^2+PG.CG)}{10{,}000}$ (index II) (X_1) and age (weeks) (X_2)	$Y = 9{\cdot}761X_1 - 0{\cdot}128X_2 + 54{\cdot}1$	146,579	38,913	12

The addition of age (weeks) as a second independent variate in these regressions (Table 7) produced significant reductions in residual variance, but the latter were relatively small, amounting to 12–21% of the residual variance after the original regressions.

Comparison of logarithmic and body volume index methods. Since it is not possible to make a direct comparison between the standard errors from regression of weight and its logarithm, fiducial limits for several of the methods were calculated and are shown in

Table 8 for estimated live weights of 875 and 1250 lb. These limits include 95% of the true values of weights estimated to be 875 or 1250 lb. The appropriate values of *CG*, *PG*, *LB* and age for estimating each of these live weights were calculated by successive substitution in the regression equations of log live weight upon log *CG*; log *CG* and log *PG*; log *CG*, log *PG* and log *LB*; and log *CG*, log *PG*, log *LB* and log age. The same values were used to estimate weights from body volume indices, with very good agreement as shown in the table.

Table 8. *Fiducial limits for live weights of* 875 *and* 1250 *lb. estimated by different methods*

Method:	Regression of log live weight on				Regression of live weight on			
	Log *CG*	Log *CG* and log *PG*	Log *CG*, log *PG*, log *LB*	Log *CG*, log *PG*, log *LB* and log age	Index I	Index II	Index I and age	Index II and age
A. Estimated live weight = 875 lb.; *CG* = 67·5 in.; *PG* = 81·1 in.; *LB* = 53·0 in.; age = 247 weeks								
Upper limit	958	946	935	930	963	955	957	949
Estimated weight	875	875	875	875	880	880	881	882
Lower limit	797	809	819	823	797	805	805	815
Upper limit–lower limit	161	137	116	107	166	150	152	134
B. Estimated live weight = 1250 lb.; *CG* = 78·3 in.; *PG* = 93·1 in.; *LB* = 57·5 in.; age = 346 weeks								
Upper limit	1372	1352	1336	1328	1338	1320	1331	1317
Estimated weight	1250	1250	1250	1250	1255	1245	1255	1250
Lower limit	1139	1156	1169	1176	1172	1170	1179	1183
Upper limit–lower limit	233	196	167	152	166	150	153	134

The differences between the upper and lower fiducial limits shown in Table 8 for logarithmic estimates were appreciably greater at the higher live weight, due to the relatively constant logarithmic error being equivalent to an increasing range of live weight as the latter increases. The addition of log *PG* and log *LB* to log *CG* reduced the range between the upper and lower limits by 28%, while the further addition of log age produced a slight further reduction of 6%. Index II, using *LB*, *CG* and *PG*, was slightly more efficient than index I, and the efficiency of the latter was still slightly less than that of the former when age was added as a second variate. The addition of age to index II reduced the range between the upper and lower limits by 11%. Taking both live weights together, both index methods were more efficient than log *CG* alone in estimating live weight. The range between limits for log *CG*, log *PG* and log *LB* was 34 lb. less at 875 lb. and 17 lb. more at 1250 lb. than that for index II using the same body measurements, i.e. the over-all difference was small and favoured the logarithmic method. A similar result was obtained when age was added to the logarithmic estimates and those from index II.

DISCUSSION

The residual variances from regressions of log live weight upon the logarithms of single body measurements confirm the results previously obtained elsewhere (7, 9, 11, 12, 13), showing that chest girth is the most suitable single body measurement for estimating live weight.

It has also been shown that the inclusion of other body measurements in addition to *CG* has appreciable effects in reducing errors of estimation of live weight. This is contrary to the previously accepted view[9,11] that such a process cannot appreciably increase the accuracy of estimation. The latter conclusion appears to have been based on the relatively small effects observed on multiple correlations when further variates were added, whereas those of the present study are based on the observed effects upon residual variance and fiducial limits of estimated weight. Since the correlation usually observed between log chest girth and log weight is well over +0·9 it is obvious that it can be increased but little by the inclusion of further measurements, but that the effects of these may be much more apparent when expressed in terms of residual error.

It has also been recommended elsewhere[11,12] that age and body condition (i.e. fatness) should be considered in making estimates of live weight from chest girth. Condition is a subjective measurement and age is not always known, so that it would obviously be preferable to use objective measurements if possible. The results of this study show that the inclusion of length of body and paunch girth had appreciable effects in reducing errors of estimation, but that the further inclusion of age had relatively minor, although usually significant, effects. The smallness of the effects of age independent of the other measurements is illustrated by the regression coefficients in Table 7, which show that its average effect was approximately −0·1 lb./week or −5 lb./year.

The comparison of body volume index methods with logarithmic methods of estimation shows that there is a very slight difference in favour of using log *CG*, log *PG* and log *LB*, rather than index II,

$$\left(\frac{LB(CG^2+PG^2+CG.PG)}{10{,}000}\right).$$

However, the latter appears to be the more practical method because of the simplicity of the calculations involved.

The error involved in estimating weight from body measurements is, of course, higher than that using one weight determined directly. The standard error of a single live weight taken at a fixed time of day is approximately 1 % of the live weight[9,11,24]. The fiducial limits for such weights recorded on cows weighing 875 and 1250 lb. will be approximately 893–857 and 1275–1225 lb. respectively, giving a range between limits of 36 and 50 lb. These may be compared with the ranges of 161 and 233 lb. for the regression of log live weight on log *CG* and, of 116 and 167 lb. for regression on log *CG*, log *PG* and log *LB* given in Table 8. The fiducial limits associated with estimation from log *CG* are therefore 4–5 times as great as those of direct determination by weighing the cow, and the inclusion of two additional body measurements reduces this ratio to just over 3:1.

SUMMARY

1. Data from 133 Dairy Shorthorn cows in three herds have been used in a study of various methods of estimating live weight from body measurements based on logarithmic relationships and indices of body volume.

2. Regression equations are presented relating live weight to several combinations of chest girth, paunch girth, length of body and age.

3. The most satisfactory estimate of weight from a single body measurement was given by the regression of log live weight upon log chest girth.

4. Appreciable increases in the efficiency of estimation were obtained by including log paunch girth or log length of body as second variates in the regression, and a further increase by including them together.

5. Indices of body volume using chest girth and length of body, or chest girth, paunch girth and length of body gave good estimates of live weight, and there was very little difference in efficiency of estimation from that when the three measurements were used in a logarithmic regression.

6. The effects of the addition of age to regressions involving chest girth, paunch girth and length of body were slight but statistically significant.

7. The most satisfactory practical method of estimation using the three body measurements is given by the equation

Live weight (lb.) $= 0{\cdot}000935\ LB(CG^2 + PG^2 + PG.CG) + 57$ (all measurements in inches).

8. Comparison of fiducial limits showed that these covered a range 4–5 times as great for live weights estimated from chest girth than for live weights determined directly, and that the inclusion of length of body and paunch girth reduced this range by 25–30%.

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663. THE RELATIONSHIP BETWEEN MILK SECRETION AND THE RATE OF MILKING BY MACHINE

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Lactation has two phases: the continuous process of secretion and the mechanism of ejection which occurs only during milking. Because ejection is a transitory phase it is to be expected that within certain limits milk yield will be inversely related to the duration of milking, which was, in fact, demonstrated many years ago with hand-milked cows (1). With machine milking the situation is different because the forces which the machine applies to the udders of all cows within a herd are the same, and thus the differences in milking rate that occur are due to differences between the cows. Under these conditions, if milking rate can be shown to affect milk yield it is a demonstration of how the physiological factors controlling milking rate can modify the expression of the inheritance of milk secretion.

The machine milking-rate and milk-yield records of eighty-six first lactation heifers presented by Dodd & Foot (2) indicated that a significant relationship existed between milking rate and lactation yield, persistency and lactation length. Since these results were published milking-rate and milk-yield records have been obtained from a further 266 first-lactation heifers and an analysis of these data is given in this paper.

SOURCES OF DATA

The records used in this analysis were obtained from three sources: heifers in the dairy herd at the National Institute for Research in Dairying and heifers at the two Bull Progeny Testing Stations maintained by the British Oil and Cake Mills Ltd. at Barlby Farm, Selby, and South Cathkin Farm, Glasgow.

Milking-rate records were normally collected for all heifers in the Reading herd in the fifth week of lactation. Although some of these animals were used in feeding trials, which may have affected lactation yields slightly, it is unlikely that this would bias the results of this analysis.

At the two Bull Progeny Testing Stations maintained by the B.O.C.M. Ltd. the first lactation performances of groups of ten to fourteen daughters of each of four bulls are compared under controlled feeding and management conditions. Daughters of each bull, from widely separated commercial herds, are moved to the Bull Progeny Testing Stations before calving. During the first lactation they are stall-fed in cowsheds and rationing of all feedingstuffs is based on the yield of milk and its chemical composition in accordance with current feeding standards.

The animals at the progeny testing stations are selected in that they are the progeny of specific bulls, but there is no reason to believe that this invalidates the results of the analysis of the milking-rate and milk-yield records of these heifers.

Milking-rate records were collected for the Friesian heifers at the Barlby Farm Bull

Progeny Testing Station in 1953, 1954 and 1955, and for the Ayrshire heifers at the South Cathkin station in 1954 and 1955.

The Friesian heifers at Selby in 1953 were milked thrice daily, otherwise all the heifers were milked twice daily. Bucket-milking units were used at both the Bull Progeny Testing Stations; at the N.I.R.D. the heifers were milked in a milking parlour fitted with pipeline recorder milking plant. Extruded teat cup liners (Alfa–Laval type no. 20003 B) were used on all three farms and the pulsators set to give 50 cyc. min. at a 1:1 ratio. At the Reading and the Barlby Stations the milking machine vacuum level was 15 in. Hg and at the South Cathkin Station it was 13 in. Hg. Milking methods on all three farms were good; udder washing preceded milking by not more than 3 min. and with only two units to operate, each milker had ample time for machine stripping.

METHODS OF RECORDING

The lactation yield of each animal was calculated from the individual weighed quantities obtained at each milking. The fat and solids-not-fat (S.N.F.) data were calculated from estimations of the composite samples taken on one day each week at the Bull Progeny Testing Stations and one day each month at Reading. The fat percentage of the milk was measured by the Gerber method and the total solids determined gravimetrically.

The method and equipment used to measure milking rates in the dairy herds at Reading have been fully described in a previous paper (4). Records were obtained at three consecutive morning milkings in the fifth week of lactation and the mean values from these records used in the following analyses.

In 1953 and 1954 an electronic recording device developed at the N.I.R.D. was used to measure milking rate at the Bull Progeny Testing Stations. The milking machine bucket was suspended by means of a hook from a strain gauge cell connected to the recording instrument which traced a graph on a moving graduated chart indicating the weight of milk in the bucket. Rate of milking measurements were extracted from the three graphs obtained for each heifer and the mean values used in the analysis. The milking-rate records of the heifers at each of the Bull Progeny Testing Stations were collected within a period of 10–14 days by a member of the staff of the N.I.R.D., and since calvings were spread over a period of 2–3 months it was not possible to confine the recording of milking rate to the fifth week of lactation. The number of days from calving when milking rate measurements were started varied from 19 to 78, with a mean of 46·9.

The definitions of the terms peak flow, standard lactation yield, lactation fat and S.N.F. percentages given previously (2) apply to the new data, except that the standard lactation length at the Bull Progeny Testing Stations is 270 instead of 305 days.

RESULTS

The additional records were from 266 cows, 135 cows from Barlby farm, 100 from South Cathkin farm and 31 from the N.I.R.D. herds. The analysis has been made separately for each set of data and presented according to the farm and year of collection. The results from Reading which were published in the first paper (2) are presented as a basis of comparison and also because they are included in the weighted regression coefficients calculated from all groups.

In Table 1 the means and coefficients of variation of the peak flow, standard lactation yield, lactation fat percentage and lactation S.N.F. percentage records are given for the various samples. In each case the results from these seven samples are similar and again emphasize the marked variation in milking rate between individual cows.

Table 1. *The means and coefficients of variation of milking rate, milk yield and composition*

Data	No. of cows	Peak flow (lb./min.)		Standard lactation yield (lb.)		Lactation fat (%)		Lactation S.N.F. %	
		Mean	Coefficient	Mean	Coefficient	Mean	Coefficient	Mean	Coefficient
N.I.R.D. 1946–52	86	4·04	35·05	6768·1	24·13	3·94	9·35	8·97	2·35
N.I.R.D. 1952–55	31	5·27	29·45	7612·7	28·95	3·53	12·57	9·05	2·10
Barlby 1953	40	5·06	41·51	9312·7	26·39	3·80	10·24	8·82	2·94
Barlby 1954	43	5·77	34·89	8826·1	19·11	3·68	8·21	8·75	2·91
Barlby 1955	52	5·00	34·33	7092·6	25·17	3·89	8·43	8·79	3·04
S. Cathkin 1954	48	4·64	31·20	7683·9	18·82	3·94	6·82	9·02	2·95
S. Cathkin 1955	52	5·29	28·30	7809·7	21·61	3·89	7·34	9·25	2·38
All data	352	—	35·61	—	25·78	—	11·21	—	3·68

Table 2. *The regression coefficients of standard lactation yield (lb.), lactation fat percentage and lactation solids-not-fat percentages on peak flow (lb./min.)*

Y	*X*	No. of cows	Farm	Regression coefficient *byx*	Correlation coefficient
Standard lactation yield (lb.)	Peak flow (lb./min.)	86	N.I.R.D. 1946/52	+418·9±117·2**	0·363**
		31	N.I.R.D. 1952/55	+386·3±254·0	0·272
		40	Barlby 1953	+509·6±170·6**	0·436**
		43	Barlby 1954	+291·1±122·6*	0·348*
		52	Barlby 1955	+277·5±141·7	0·267
		48	S. Cathkin 1954	+147·3±145·6	0·148
		52	S. Cathkin 1955	+601·8±134·7**	0·534**
		352	Weighted mean	+381·4± 55·7**	0·349**
Lactation fat (%)	Peak flow (lb./min.)	86	N.I.R.D. 1946/52	−0·0348±0·0282	—
		31	N.I.R.D. 1952/55	+0·0571±0·0522	—
		40	Barlby 1953	−0·0186±0·0298	—
		43	Barlby 1954	+0·0449±0·0223	—
		52	Barlby 1955	+0·0004±0·0147	—
		48	S. Cathkin 1954	+0·0509±0·0263	—
		52	S. Cathkin 1955	−0·0306±0·0636	—
		352	Weighted mean	+0·0044±0·0135	—
Lactation S.N.F. (%)	Peak flow (lb./min.)	86	N.I.R.D. 1946/52	−0·0411±0·0162*	—
		31	N.I.R.D. 1952/55	−0·0274±0·0222	—
		40	Barlby 1953	−0·0580±0·0177**	—
		43	Barlby 1954	+0·0133±0·0197	—
		52	Barlby 1955	−0·0061±0·0219	—
		48	S. Cathkin 1954	−0·0025±0·0268	—
		52	S. Cathkin 1955	−0·0099±0·0207	—
		352	Weighted mean	−0·0200±0·0078*	—

* Significant when $P<0·05$; ** significant when $P<0·01$.

In Table 2 the regression coefficient of standard lactation yield, lactation fat percentage and lactation S.N.F. percentage on peak flow are given for each of the seven sets of data, together with a weighted mean of all results. A number of the more relevant correlation coefficients are also given.

The seven regression coefficients of standard lactation yield on peak flow are all positive, though only four are statistically significant with a fifth value almost significant. The weighted mean regression coefficient calculated on the complete data is very highly

significant. This direct relationship between inherent milking rate and yield in the first lactation confirms the earlier results (2).

Six of the seven regression coefficients of lactation S.N.F. percentage on peak flow are negative and two are statistically significant. The weighted mean regression coefficient calculated on all the data is negative and significant.

In the original paper (2) it was stressed that it is impossible to say with certainty which are the dependent factors in these relationships. With the regression of milk yield on milking rate there is little doubt that to some degree each is dependent upon the other.

However, there is evidence (5) that milking rate is closely related to the effective size of the teat orifice during milking and only to a much less degree affected by milk yield. In addition, because persistency and lactation length are also related to milking rate it would appear that the main relationship is that of milk yield on milking rate. Nevertheless, since it has been shown that milk yield does affect milking rate (4), particularly with fast milking cows, a further analysis has been carried out.

Records are available for 135 animals with mean yields within the limits of 15·0 and 17·9 lb. milk on the three recording days. The linear regression coefficient of lactation yield (y) on peak flow (x_1) has been calculated for these animals, together with the multiple regression coefficient where correction was made for the milk yield at the milkings when the recordings of rate of milking were made (x_2). The linear regression coefficient (byx_1) is 344 ± 80 and the multiple regression coefficient ($byx_{1.2}$) 326 ± 81. These values are almost identical and similar to the mean regression given in Table 2. This indicates that the differences in yield at recording do not bias the results to any marked degree.

DISCUSSION

These results lend support to the original data which indicated a relationship between milk yield and milking rate and strengthen the belief that milk yield is dependent to some degree on the rate of removal of milk from the udder.

It is clear from the levels of the correlation coefficients that selection on the basis of milking rate as a means of improving milk yields would be of very little value, quite apart from the practical difficulties of measuring milking rate. In practice, involuntary culling on the basis of milking rate will occur even when its direct measurement is not made (2).

Furthermore, the use of progeny testing indices, calculated solely on the milk yields of a bull's daughters, will automatically select for milking rate, providing that all available daughters are included in the calculation. It is particularly important that animals with short lactations are not excluded since our evidence indicates that these are likely to be the slower milkers. When bulls are being tested in a progeny testing station the measurement of milking rate is very useful additional information.

The slight relationship between milking rate and the S.N.F. percentage mentioned in the earlier publication (2) is again demonstrated in Table 2.

We would like to thank Mr J. C. Leslie, O.B.E., of British Oil and Cake Mills Ltd. for permission to collect and use the records from the progeny testing stations, and also the two farm managers, Mr P. Bichan and Mr W. Dunbar. We are indebted to Miss E. J. Goulding and Mr R. Phillips for help in the analysis and presentation of the results.

ADDENDUM

Brumby (6) has recently published a preliminary analysis of the relationship between maximum daily yield and maximum milking rate from data collected from 110 cows. He found no significant relationship and suggests that the differences between his results and those published previously (2) are due to differences in milking methods. It is to be expected that the effect of milking rate on milk yield will be greater under poor milking conditions. However, both the milking equipment and the milking routines at the N.I.R.D. and the progeny testing stations can be classified as good.

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664. THE EFFECT OF THYROTROPIN ON MILK YIELD AND HEART RATE OF THE GOAT

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(With 1 Figure)

Earlier experiments from this Institute have shown that thyroxine, when administered to lactating cows and goats, causes a number of well-defined changes in the composition of the milk (1,2,3,4). In particular, it was found (4) that thyroxine increased the vitamin A ester and carotene in the milk when the diet contained carotene, and that it increased the vitamin A alcohol when the diet did not contain carotene. More recently, Chanda, Clapham & Owen (5) found that 'Ambinon', which is a pituitary preparation containing thyrotropin together with the follicle-stimulating and luteinizing hormones, can simulate these effects of thyroxine on the metabolism of carotene in the cow. In these experiments a somewhat surprising result was that, unlike thyroxine, the Ambinon did not increase either the yield of milk or the ratio of phosphorus to nitrogen in the milk (1,2).

There were some features of difference between the Ambinon and the thyroxine experiments which might account for this. The first and most obvious one was that whereas the maximum safe daily dose of thyroxine for a cow was known (1), the maximum safe dose of a thyrotrophic preparation was not known. Secondly, thyroxine had been injected daily, but the thyrotrophic preparation had been given only on alternate days. Thirdly, in view of the work of Gyllensten & Olsson (6) there was the possibility that thyrotropin might to a considerable extent be inactivated in lymphatic tissues before it reached the point in the body (presumably the thyroid gland) where it could produce its physiological effects. It was therefore thought advisable to study the effects of larger and more frequent doses of thyrotropin to see if they would increase the yield of milk.

For comparison of galactopoetic effects, growth hormone was also used.

EXPERIMENTAL

Hormones used. These were 'Somatotropin' and 'Thyrotropin' of Armour Laboratories (Chicago, Illinois). They were received by air from the U.S.A. as freeze-dried pure white powders in sterile, rubber-capped, multidose, self-sealing vials. The 'Thyrotropin' contained 40 United States Pharmacopoea units per vial. Each 'Somatotropin' vial contained the equivalent of 50 mg. of the Armour growth hormone standard. The hormones were brought into solution by injecting a measured amount of sterile distilled water through the rubber seal with a graduated syringe. Between dosings the vials were kept in a refrigerator at 2–4° C.

Plan of experiment. The production ration throughout the experiment consisted of a mixture of 3 parts by weight of crushed oats and 2 parts of bean meal. In the first period of the experiment, each goat received 3 lb. of this mixture daily in two equal portions, one at the morning milking and one at the evening milking. As roughage the goats were

allowed to eat oat straw to appetite. The experiment was divided into periods 1, 2 and 3, of duration 24, 10 and 12 days respectively (Table 1). Five British Saanen goats were used. During period 1 all five goats were treated alike, but during period 2, two of them each received daily, by subcutaneous injection into the flank, 10 mg. Somatotropin; two others each received daily, by subcutaneous injection, 2 U.S.P. units Thyrotropin. On the fourth day the doses of both hormones were doubled and these doses were continued for a further 7 days. A fifth goat received the same dietary treatment as the other four but was given no hormones. At the beginning of period 2 the concentrate ration of all the goats including the control goat was increased to 4 lb. daily, so that limitation of food intake would not limit their capacity to respond to the hormone treatment. During period 3, the effects of recovery from the hormone treatments were studied.

Table 1. *Average milk yields (g. per goat per day) and effect of treatment as measured by the difference between actual and expected yields*

					Periods 2 and 3 together		
Name of goat	Hormone treatment in period 1	Period 1 (24 days)	Period 2 (10 days) (a)	Period 3 (12 days) (b)	Actual yield ($d = \frac{1}{22}(10a+12b)$)	Expected yield (y)*	$d-y$
Avril	Growth hormone	1156	1687	1566	1621	1031	+590
Maisie	Growth hormone	1800	1952	1928	1939	1486	+453
Jane	Thyrotropin	1215	1340	1374	1359	701	+658
Rainbow	Thyrotropin	1004	1041	1044	1043	965	+ 78
Sunshine	None	1286	1328	1244	1282	1135	+147†

* The yield that would have been expected, had no hormone been given, was calculated as the intercept on the mid-ordinate of periods 2 and 3 taken together, cut off by the straight line fitted by the method of least squares to the daily yields in period 1.

† The increase of the control animal above expectation is due to the fact that the food allowance was increased from 3 to 4 lb. per goat per day at the beginning of period 2 (see text).

RESULTS

Milk yields. The effects of the hormone treatments on the milk yields can be seen in Fig. 1. Numerical estimates of the effects of the hormones on the milk yields were made by fitting a straight line, by the method of least squares, to the pretreatment daily milk yields for each goat. Production of this line on the graph (Fig. 1) through the treatment and recovery periods gave a measure of what the milk yield would have been had no hormone treatment been given. Expected yields, so calculated, are compared with the actual yields in Table 1 which shows that, by comparison with the control animal, the two goats which had received growth hormone had had their milk yields increased and that there was an increase in the yield of one of the thyrotropin goats.

A second numerical estimate of the effects of the hormones was made by calculating the average rate of increase of the milk yields during period 2. This was done by calculating the slope of the straight lines fitted by the method of least squares to milk yields during period 1, during period 2 and during period 3. The results of the calculations (Table 2) show that the rates of change in period 1 were negative for all the goats and became positive in period 2, the increase of rate being greatest in the two growth hormone goats and one of the thyrotropin goats. The remaining thyrotropin goat, Rainbow, showed a change of about the same size as that of the control animal. The positive response of the control animal itself was due to the fact that, at the beginning of period 2, the allowance

of meal to all the goats was increased from 3 to 4 lb. per day. Table 2 also shows in its last two columns that in all the treated goats the rate of decrease of milk yield on discontinuance of the hormone treatment was much greater than the rate of decline shown by the control goat.

Heart rates. In Table 3 the initial heart rates, and the rates during treatment and recovery are shown together with the rates of change of the heart rates during treatment

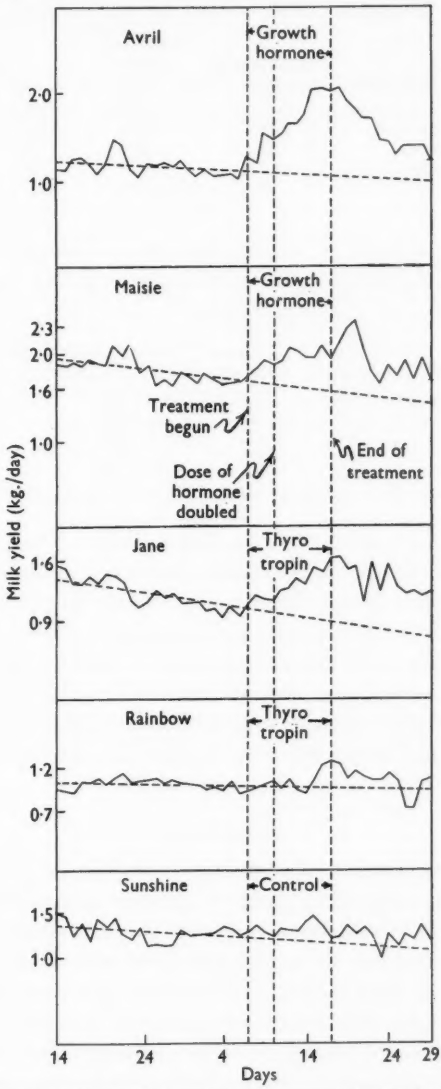

Fig. 1. Milk yields of goats treated with growth hormone or thyrotropin depicted with the yield of the untreated goat, Sunshine, on the same rations. At the first dotted ordinate treatment was begun, at the second doses of hormone were doubled, at the third treatment ended. The sloping line, which was fitted by the method of least squares to the pretreatment yields, was used as an index of the yield to be expected in periods 2 and 3 taken together.

and during recovery. The heart rates increased in all the treated animals, but the increase shown by Maisie was small. The control animal's pulse showed a small decrease from 106 to 101 beats per min. during the treatment period. Rates of change of pulse during treatment were calculated as regression coefficients by the method of least squares. These are

Table 2. *Rate of change of milk yield expressed as increase (+) or decrease (−) in grams of milk secreted per goat per day*

Name of goat	Hormone treatment	Initial period (*a*)	Treatment period (*b*)	(*b* − *a*)	Recovery period (*c*)	(*c* − *b*)
Avril	Growth hormone	− 5·2	+90·5	+95·7	−69·7	−160·2
Maisie	Growth hormone	−13·1	+29·7	+42·8	−35·9	−65·6
Jane	Thyrotropin	−21·4	+44·7	+66·1	−34·7	−79·4
Rainbow	Thyrotropin	− 1·7	+14·5	+16·2	−25·2	−39·7
Sunshine	None	− 6·3	+12·9	+19·2	− 2·1	−15·0

Table 3. *Heart rates (pulses per minute) and rates of increase (+) or decrease (−) of heart rates during treatment with hormones and recovery from the treatment*

Name of goat	Treatment	Average pulse rate: Initial period	Average pulse rate: Treatment period	Rate of change during treatment	Average pulse rate recovery in period	Rate of change during recovery
Avril	Growth hormone	96	127	+4·5	127	−2·0
Maisie	Growth hormone	90	97	+0·7	102	−0·7
Jane	Thyrotropin	100	113	+4·4	116	+0·4
Rainbow	Thyrotropin	108	120	+3·0	130	−2·5
Sunshine	None	106	101	+0·9	108	−0·7

recorded for both treatment and recovery periods in Table 3. Judged both by pulse rates and by rates of change of pulse rates, Maisie's heart showed changes of rate almost the same as those of the control animal. The other three goats showed greater accelerations of heart rate during treatment and greater decelerations during recovery. In themselves these changes of heart rate are perhaps not remarkable. Their importance is that they are in marked contrast to the very large increases of heart rate (1) which result when thyroxine is given, and that they raise the question as to how much, if any, of the effects of these two hormones on the milk yield is mediated by the animal's own thyroid gland.

DISCUSSION

After this experiment was planned, but before it was begun, a paper by Wrenn & Sykes (7) appeared. It showed that Thyrotropin from the Armour Laboratories could stimulate the yield of milk by cows. In the present experiments the initial daily doses were calculated from those of Wrenn & Sykes (7) by assuming that the ratio of the effective dose of each hormone in the goat to the dose of each hormone in the cow would be equal to the ratio of their respective body weights raised to the power 0·75 (8). As will be seen from Fig. 1, these initially calculated doses when doubled produced the increases of yield here reported. The goats which received Thyrotropin showed no ill effects at any time during the experiment, and it is quite reasonable to suppose that the Thyrotropin would have caused an even larger stimulation of yield had a larger dose been given.

SUMMARY

1. The effects of thyrotropin on milk yields and heart rates of goats were compared with the effects of growth hormone.

2. As expected, the milk yields from the goats which received growth hormone were markedly increased.

3. Thyrotropin caused increases of heart rate in two goats in one of which a large increase of milk yield was also produced.

The authors wish to thank Dr Irby Bunding of Armour Laboratories, Chicago, Illinois, U.S.A., for a gift of the hormones used in these experiments.

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665. MAMMARY GLAND DEVELOPMENT IN THE CALF AS A POSSIBLE PREDICTOR OF SUBSEQUENT MILK PRODUCTION

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A report from the United States Department of Agriculture in 1947 (1) claimed that a measurement of the mammary gland development of the calf could give some indication of the animal's future milk production. Although the first report did not contain full experimental results, the few figures that were published, and the very strong recommendation from the authors that the relationship should be tested further, encouraged the launching of a field trial at Reading University in 1950.

Since 1950 there have been further reports from the U.S.A. and a report from Ruakura Animal Research Station in New Zealand. The United States Department of Agriculture experiment was not fully reported until 1955 (2), by which time 280 Holsteins and 222 Jerseys had provided records over 21 years. The correlation coefficients between their mammary development as calves and their subsequent milk yield proved disappointing, being 0·2733 for the Holsteins and 0·1877 for the Jerseys. Other reports from the U.S.A. (3, 4, 5) have shown little, if any, relationship, and New Zealand workers (6) have also concluded that there is no useful correlation.

EXPERIMENTAL

Method

A palpation technique for the assessment of udder size in the 4-month calf has been described by Swett in detail (2). This method was followed exactly and resulted in measurements of width, length and stage of each developing quarter of the calf mammary gland. In addition, the heart girth of every calf was measured. All the measuring was done by one person and covered an intensive period of 7 months.

Material

A request to co-operate in the study was made to all farms within 60 miles of Reading with more than 100 cows milk-recorded by the Milk Marketing Board. Thirty-four farms agreed to co-operate, providing a potential 1190 calves for measuring, but during the measuring period six of these farms were abandoned as unsatisfactory. On the remaining twenty-eight farms, 727 calves were measured at the age of 4 months or 1 week either side. The measurements were taken between December 1950 and June 1951.

First lactation records were obtained for 485 of these 727 calves. However, after eliminating all those heifers which did not calve within a few months of 3 years old (June 1953 to February 1954 inclusive), all those giving lactations of under 200 days, and all herds finally contributing less than five measured animals, the final number to be subjected to analysis was 256. Of these 256 animals, 169 were Dairy Shorthorns in seventeen herds,

75 were Ayrshires in seven herds, and 12 were Friesians in two herds. Only the data from the 169 Shorthorns and 75 Ayrshires were fully analysed. Since only seven of the farms remaining in the final analysis were fat-tested, no analysis of fat yields was made.

RESULTS

The total between- and within-herd correlation coefficients between each of the three measurements of udder development and milk yield, and between heart girth and milk yield, are shown in Table 1.

Table 1

	D.F.	Correlation coefficients of milk yield with: Width	Length	Stage	Heart girth
Shorthorns					
Total	167	0·005	0·060	−0·054	0·469**
Between-herds	15	−0·174	−0·022	−0·024	0·663**
Within-herds	152	0·104	0·108	−0·072	−0·018
Ayrshires					
Total	72	−0·077	0·053	0·039	0·189
Between-herds	6	−0·483	−0·403	−0·151	−0·294
Within-herds	66	0·076	0·222	0·085	0·424**

** = Significant at the 1% level. All unmarked correlations are non-significant. D.F. = degrees of freedom.

These correlation coefficients are very low indeed and there can be little doubt that under the conditions of the experiment there is no relationship between the measured mammary gland development of these calves and their subsequent milk yield.

The coefficients of variation of the five factors are shown in Table 2.

Table 2

	Yield	Width	Length	Stage	Heart girth
Shorthorns					
Total	26	42	48	25	4·2
Between-herds	60	69	74	34	8·3
Within-herds	19	39	45	24	3·6
Ayrshires					
Total	20	31	42	16	5·6
Between-herds	34	61	80	22	12·6
Within-herds	19	27	36	15	4·5

DISCUSSION

The results from this field trial are quite conclusive. It can be said with confidence that under the conditions of the experiment there is no relationship between calf mammary development and subsequent first lactation milk production.

Though the conditions of the trial have ensured that the conclusions are applicable to the normal commercial dairy farm, the experiment has two definite limitations. No allowance has been made for the effect of feeding on calf mammary development. Secondly, the udder development has been measured at only one age. As a subsidiary investigation has shown that the predictability of udder development from one age to another is probably low, the experiment has not eliminated the possibility of a useful correlation at another age.

The justification for further investigation, with the correction of these two faults in mind, rests on reports from other parts of the world as to the importance of these limitations. The majority of these other investigations have, however, been undertaken to test the practical application of the hypothesis under conditions similar to those found at Reading. The limitations are the same, the results have been very similar, and the conclusion that no correlation exists under these conditions has been universal. The justification for further work on the subject therefore still rests with the original investigators, the United States Department of Agriculture, and on their results from the Beltsville herd. The size and the supreme management of this herd make it unrivalled as a testing ground for such a theory, and the results are free from the two particular faults of the Reading experiment. Those results do not, however, indicate a sufficiently valuable relationship between mammary development and subsequent milk yield to suggest the existence of a useful correction in even the most suitable herds in this country.

CONCLUSION

It is concluded that under normal dairy farm conditions in this country there is no relationship between mammary gland development in the calf and subsequent first lactation milk yield. Further investigation in the hope of a successful application to selected herds, or in the hope of finding a correlation for the effect of environment in the normal herd, is not justified by any results yet published.

Acknowledgement is due to the University of Reading for a Research Grant during the tenure of which this work was undertaken; to Prof. H. G. Sanders and Mr S. Fox for the direction of the study; and to the owners of the farms on which the calves were measured.

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636.32:612.664.3

666. INTRAMAMMARY PRESSURE CHANGES IN THE LACTATING EWE

I. THE NATURE OF THE PRESSURE CHANGE UNDER STANDARD CONDITIONS

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(With 8 Figures)

There appear to be considerable species differences between mammals with respect to the intramammary pressure change which occurs during the ejection of milk. One of the earliest pressure/time curves published was that recorded by Gaines (1) for the lactating goat. This is reproduced in Fig. 1. Earlier studies on the sow at this station (2,3) have shown that a different sort of pressure change is encountered in the sow compared with the goat. A typical sow graph is shown in Fig. 2. It is characterized by a sharp rise in pressure followed by an immediate but slower fall to pre-ejection level. The pressure response of the cow is different from either the goat or the sow (4). The onset of milk ejection in the cow is shown by a sudden increase in pressure. In the unmilked gland this pressure shows only a slight fall over a period of about 20 min.

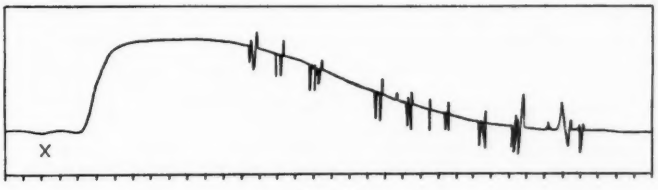

Fig. 1.

In order to obtain additional data which may help in clarifying the reasons for these differences in response, it was thought worth while to study the pressure changes occurring in the ewe under various conditions. This paper describes the response of the ewe to standard doses of oxytocin. The apparatus used for the work is described in the Appendix.

METHODS

The ewes were separated from their lambs for 3 hr. prior to the experiment. They were brought into a milking bail and a teat cannulated, using the same type of cannula as that used earlier for sows (2). All injections were made into the jugular vein.

Fig. 3. shows the pressure response of a ewe injected with five doses of 0·25 i.u. of oxytocin at intervals of 6 min. The interval between injection and let-down and the

pressure rise are set out in Table 1. In this case the ewe was not milked prior to the experiment, so that the milk cistern and larger ducts contained milk—a situation characteristic of the ewe.

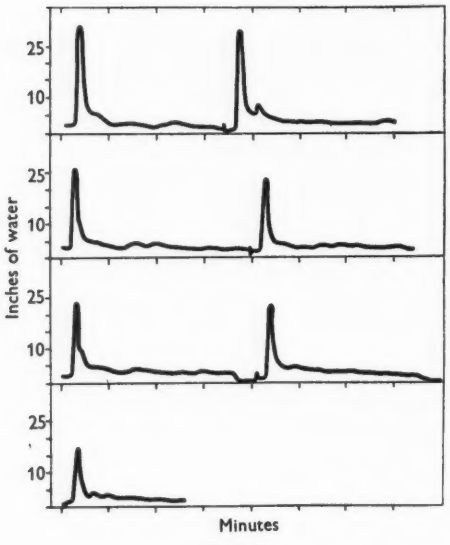

Fig. 2.

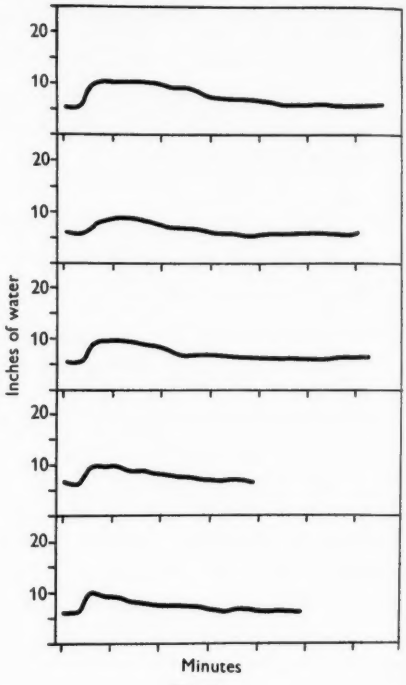

Fig. 3.

Table 1

Dose of oxytocin (i.u.)	Interval between injection and ejection (sec.)	Presure rise (inches of water)	Ewe number	Figure number
0·25	22·8	5·5	1550	3
0·25	25·8	3·3	1550	3
0·25	24·0	4·5	1550	3
9·25	22·8	4·0	1550	3
0·25	22·8	4·5	1550	3
0·5	38·4	7·0	1550	4
0·5	18·0	5·0	1550	4
0·5	18·0	3·9	1550	4
0·5	13·2	17·2	1526	5
0·5	12·0	8·0	1526	5
0·5	12·0	8·0	1526	5
0·25	15·6	7·3	1549	6
0·5	16·2	4·6	1549	6
0·25	18·0	2·5	1549	6
1·0	17·4	4·0	1549	6

Fig. 4 illustrates the response of the same ewe to three successive doses of 0·5 i.u. of oxytocin. Again, the ewe was not milked prior to recording the pressure change. The delay between the first injection and the rise in pressure is abnormally long in this case. The reason for the delay is not known.

In Fig. 5 the ewe was milked out by machine before measuring the pressure changes. Three 0·5 i.u. doses of oxytocin were given at 12 min. intervals. The ewe used in this experiment was rather restless, with the result that small variations in pressure occurred because of the animal's movements.

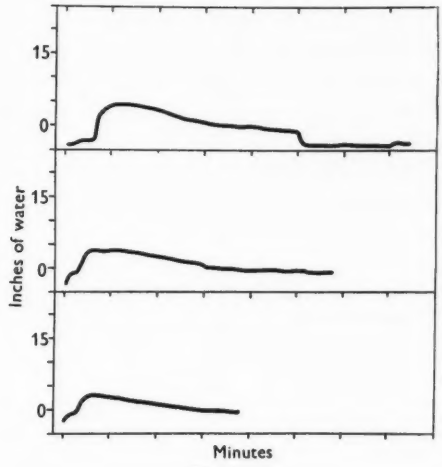

Fig. 4.

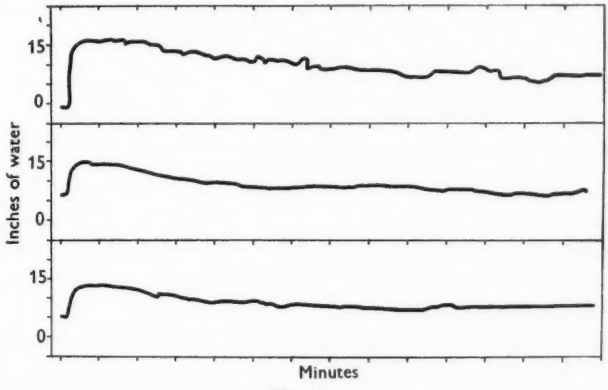

Fig. 5.

The four pressure graphs given in Fig. 6 were produced by the following series of injections: 0·25, 0·5, 0·25, 1·0 i.u. of oxytocin.

DISCUSSION

The shape of the ewe intramammary pressure curve for the unmilked gland is quite different from that given by the cow and the sow. It is, however, similar to the curve recorded by Gaines (1) for the goat. The ewe pressure curve is characterized by a rapid rise rounding off to a plateau, followed by a steady fall. Many curves show a short accelerated fall which is shown clearly in Fig. 3. The significance of this is not understood.

In the case of Fig. 4, when the gland was milked prior to making the recording, the final equilibrium pressure after the first injection was higher than the initial pressure. This is presumably due to the hydrostatic pressure of milk in the cistern and large ducts. It is a noticeable characteristic of the sheep that there is always a high proportion of 'cistern milk' which may be obtained before milk ejection occurs.

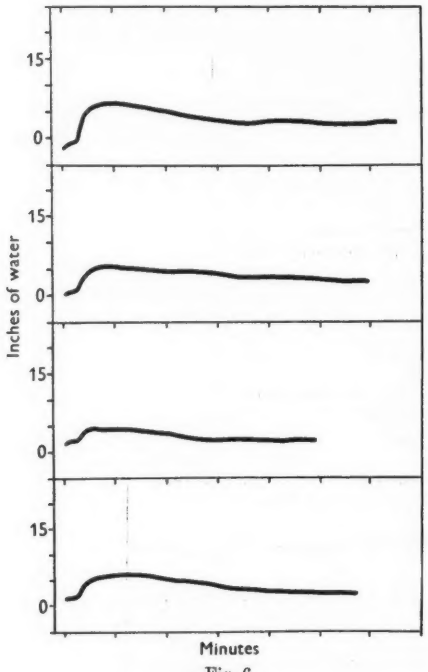

Fig. 6.

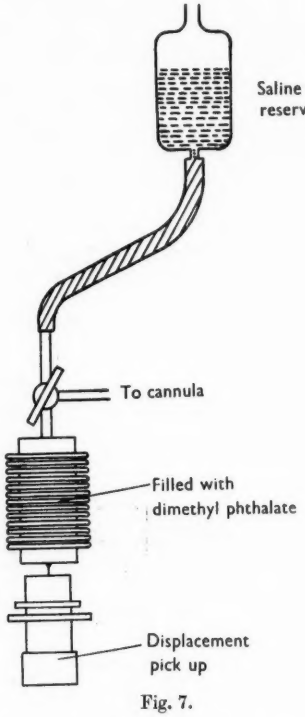

Fig. 7.

The suddenness of the ejection pressure rise is well demonstrated in Fig. 5, where the gradient of the line is 4·8 in. of water per second. This is of the same order as the initial pressure rise in the sow (Fig. 2).

Until the structural differences between species are clarified, no explanation can be offered for the differences in the milk-ejection pressure curves. The cow, sow and ewe all show a sudden pressure rise. The 'fall' phase of the curves suggests that in the cow there is a 'non-return valve' mechanism, in the ewe a high reverse flow impedance, and in the sow the ducts are low in impedance to flow both ways. A study of the comparative anatomy of the mammary glands of these animals would be most illuminating, and is essential for a complete understanding of the phenomena described above.

SUMMARY

The effect of oxytocin on the intramammary pressure of the lactating ewe has been described. The pressure curve produced by the ewe is shown to be different from both sow and cow.

An improved pressure recorder is described.

The author wishes to record his thanks for the assistance of Miss W. Wilkie and Mr E. D. O'Reilly.

APPENDIX

AN IMPROVED RECORDER FOR INTRAMAMMARY PRESSURE CHANGES

The apparatus used earlier for measuring pressure changes within the mammary gland of the sow employed a special type of mercury manometer as the measuring head. This had the advantage of being self-calibrating and also reproduced the conditions obtaining when pressure was measured with orthodox equipment. However, such equipment is fragile and in addition has the disadvantage that zero setting involves moving the manometer about with respect to the level of the gland of the animal being studied. The apparatus to be described is very robust and has provision for electrical zero setting.

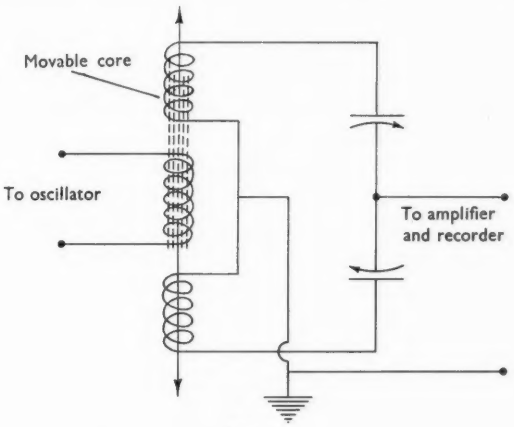

Fig. 8.

The measuring head (Fig. 7). This consists of brass bellows connected to a two-way cock which is in turn connected with a saline reservoir and the connecting cannula. As brass bellows tend to corrode in the presence of saline, the unit is filled with dimethyl phthalate —a liquid which is stable, non-corrosive, heavier than water and readily available.

The electrical circuit. The movements of the bellows are followed by a Philips magnetic displacement pick-up. This device is essentially a small transformer with two secondaries. The coupling between the primary and the secondaries is changed by the movement of a 'Ferroxcube' core in such a way that a small movement causing an increase in coupling, and therefore in output voltage of one secondary, causes a decrease in the other. If the secondary windings are connected in a bridge circuit (Fig. 8) in which the balancing arms are variable capacitors, the output voltage may be set at zero for any position of the pick-up within the measuring range and subsequent movements produce an output substantially proportional to the movement of the pick-up, and therefore, to the pressure applied to the bellows. The capacitor bridge was made by coupling two 300 $\mu\mu$fd portable radio type of variable tuning condensers together mechanically and using a radio tuning dial with planetary reduction gearing.

A Wien bridge type of oscillator supplying a few volts of 6000 c.p.s. a.c. feeds the

bridge and the output is amplified as described earlier. The final recording instrument used is an Elliott 0–2 mA. recorder which has a rectilinear chart. This instrument has been found to be thoroughly reliable and robust.

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667. SYNTHESIS OF LACTOSE *IN VITRO* BY MAMMARY GLAND SLICES FROM LACTATING RATS, GUINEA-PIGS AND SHEEP

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There can now be little doubt that glucose is the principal precursor of lactose in the mammary gland. Evidence for this comes from *in vivo* and *in vitro* studies, using both analytical and isotopic techniques (1), though considerable species differences have been noted.

Net synthesis of lactose from glucose has been conclusively demonstrated in mammary gland slices from lactating guinea-pigs by several workers (2, 3, 4), while results from similar experiments with rat tissue have, in general, been unconvincing. Heyworth & Bacon (4) carried out a number of such experiments, but could find no conditions under which lactose was synthesized, even with incubation periods of up to 6 hr. Hills & Stadie (5) claimed to have shown lactose synthesis in lactating rat mammary slices, but their lactose determinations were apparently carried out only on the medium in which the slices had been incubated. Their experimental details are insufficient to support the claim in the view of Heyworth & Bacon (4), who showed that preformed lactose may be lost from slices to medium. Caputto & Trucco (6) have shown incorporation of ^{14}C into lactose when lactating rat mammary slices were incubated in a medium containing ^{14}C-glucose. However, evidence of incorporation, though showing that the mechanisms of synthesis are present, is not necessarily evidence of net synthesis *in vitro*.

Malpress & Morrison (3) were unable to detect lactose synthesis in slices of cow udder, and in a critical assessment of previous claims concluded that no *in vitro* demonstration of lactose synthesis by the ruminant mammary gland had been made.

In the experiments reported here 0·5 g. batches of washed slices of mammary gland from actively lactating guinea-pigs, sheep or hooded Norway rats were incubated for 3 hr. at 37° in 5 ml. Krebs's bicarbonate ringer (gas phase 95% O_2; 5% CO_2). The substrates used were either (*a*) uniformly labelled ^{14}C-glucose (0·3%, w/v; 50μc mmole), or (*b*) sodium 2-^{14}C-pyruvate (0·02 M; 50μc./mmole) with or without the addition of inactive glucose (0·3%, w/v). After incubation the slices and medium were homogenized and deproteinized by addition of an equal volume of 0·3 N barium hydroxide solution, followed by an equivalent volume of 0·3 N zinc sulphate. Zero-time blanks were prepared by adding mammary gland slices to the medium containing labelled substrate, followed immediately by deproteinizing as described. Clear supernatants were obtained from blank and incubated samples by centrifuging. When labelled glucose was the substrate, these supernatants were usually used without further treatment for paper chromatography. When labelled pyruvate was used, the solutions were deionized by successive batch treatments with Amberlite IR 120 (H) and Amberlite IRA 400 (carbonate form). This procedure

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removed pyruvate without affecting lactose, whereas the hydroxyl form of IRA 400 was found to remove a large proportion of the lactose from a dilute solution (cf. Phillips & Pollard (12)). Mixed resins such as Biodeminrolit (Permutit) also were found unsatisfactory, even when treated with CO_2 as suggested by Woolf (7).

0·5 ml. volumes of the protein-free supernatants, deionized if necessary, and corresponding to about 2 mg. dry weight of mammary tissue, were applied to Whatman no. 1 paper as single spots (8) and developed by descending chromatography using 85% (v/v) acetone as solvent. Glucose and lactose standards were also run. In this system the R_F values of these sugars are low. The solvent is fast-running however, and if it is allowed to drip off the lower edge of the paper, which is serrated, an overnight run gives excellent separation of glucose and lactose, which travel about 25 and 10 cm. respectively. This simple system also has the advantage over most two-phase solvent systems that constancy of temperature is not necessary. It has satisfactorily separated raffinose, lactose, glucose, fructose and ribose.

After running, the papers were dried and placed in contact with Ilford Industrial G X-ray film for 7–14 days. The films were then developed and the papers sprayed with 2% aniline hydrogen phthalate in 95% ethanol followed by heating.

In tissue from all three species a small amount of lactose was detectable in the zero time blanks, and this was considerably increased in the samples incubated with glucose as a substrate. These results were consistently obtained in a number of experiments, and are believed to provide the first definite evidence of net lactose synthesis in mammary slices of rat and ruminant. They are supported by the finding of radioactivity in lactose of all three species when ^{14}C-glucose was the substrate. No activity was present in the zero time blanks.

Compounds reacting with aniline phthalate and running more slowly than lactose on a paper chromatogram have been reported in rat milk by Roberts, Pettinati & Bucek (9) and in extracts of rat mammary gland by Caputto & Trucco (6). Both groups of workers found them to contain glucose and galactose, and radioactivity was incorporated when rat mammary slices were incubated with ^{14}C-glucose (6). A similar compound was found in the present experiments, using rat tissue. Furthermore, net synthesis was demonstrable, and both chemical concentration and radioactivity approximated to those of the lactose, supporting the view (6) that such compounds may be connected with the formation of lactose. However, no trace of such compounds was found after incubation of mammary tissue from sheep or guinea-pigs.

Lactose from one rat experiment using ^{14}C-glucose substrate was eluted, hydrolysed and run on paper with the benzene–butanol–pyridine–water system of Gaillard (10). Radioautography and location of sugar spots showed that glucose and galactose had about the same radioactivity, confirming previous evidence (6) that the transformation glucose→galactose takes place in the mammary gland.

In tissue from all three species there was no *in vitro* incorporation of radioactivity into lactose when ^{14}C-pyruvate was the only substrate. When inactive glucose was added there was consistently detectable incorporation, but at a much lower level than from ^{14}C-glucose. There may thus be a small degree of reversibility in the conversion of glucose to C_3 units. However, it is clearly not enough to cause considerable dilution of glucose, a possibility suggested by Duncombe & Glascock (11) to account for the inhibiting effect of added pyruvate on the oxidation of labelled glucose by rat mammary slices *in vitro*.

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668. THE INFLUENCE OF CREAM RISING UPON THE ACTIVITY OF BACTERIA IN HEAT-TREATED MILK

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The problem of slow cheese starters is well known and has recently been summarized in a review by Babel (1). Some of the responsible factors such as bacteriophage and presence of antibiotics are well known, but other factors have received less detailed study.

In the course of an investigation of the conditions responsible for some cultures being slow and others fast, it became apparent that the rate of cream rising was a significant factor. Although generally known, its significance may not always have been appreciated, and in consequence the work has been extended to include the influence of cream rising on the keeping quality of pasteurized and homogenized milk.

I. ACTIVITY OF STARTER CULTURES

The activity of these organisms is commonly assessed by measuring the acidity produced in heat-treated or pasteurized milk after a suitable period of incubation, e.g. 6 hr. at 30° C. In this work the activity test has been carried out in the following manner:

10 ml. of H.T.S.T. pasteurized milk, 71·5° C. (161° F.)/15 sec. pre-warmed to 30° C., were inoculated with 1 ml. of a 1/10 dilution of a 24 hr. culture in autoclaved milk incubated at 22° C. and the tube placed in a 30° C. water-bath. After 6 hr. the acidity developed was measured by titration against N/9 caustic soda.

In order to follow the development of acidity during the 6 hr. period at 30° C., a small amount (0·004%) of Bromo–Cresol Purple (B.C.P.) was added to duplicate tubes. Fast and slow starters showed a marked difference in appearance in the tubes containing B.C.P. Whereas the colour change was uniform throughout the tube in the case of most fast starters, many slow starters caused a rapid colour change to yellow in the cream layer which forms during the 6 hr. incubation, whilst the remaining milk beneath the cream layer remained slate blue in colour throughout this incubation period. Microscopic examination showed that with fast starter cultures growth seemed to proceed uniformly throughout, i.e. both in the cream layer and the underlying milk, but that in the case of slow starters no growth appeared to take place in the milk for at least 4 hr., whereas very large numbers of organisms were found in the cream layer after 1–2 hr., and these proceeded to multiply normally during the remainder of the 6 hr. incubation.

Growth in separated and homogenized milk

Since the above observations suggest that the cells of slow starter cultures are carried up with the cream, the activities of these slow cultures were determined in separated milk and homogenized milk, in both of which the effect of cream rising is eliminated. Typical results are shown in Table 1.

As expected, higher activities were found in both types of milk. Little difference between activities was noted when fast starters were used.

Table 1

Starter no.	Pasteurized	Separated	Homogenized
803 (slow)	0·220	0·325	0·430
760 (slow)	0·230	0·360	0·420

Pasteurized, Homogenized: Pasteurized commercially — 71·5° C. (161° F.)/15 sec.
Separated: Laboratory pasteurized — 62·8° C. (145° F.)/30 min.

Effect of inversion of tubes during 6 hr. incubation

Inverting the tubes every 30 min. as in the official methylene blue test for raw milk (2) increased the acidity produced, but an even greater increase was obtained by covering the surface of the milk in the tube with wax and inverting every 30 min. The following results were obtained using mechanical means of inverting tubes in a 30° C. water-bath (3 rev./min.), all tubes being closed with rubber bungs.

Table 2

Starter no.	Milk	Normal test (without rotation)	Tubes rotating: Wax on milk	Tubes rotating: No wax
760 (slow)	H.T.S.T.	0·220	0·465	0·310
760 (slow)	Homogenized	0·425	0·495	0·295
924 (fast)	H.T.S.T.	0·620	0·645	0·295

It will be seen that the incorporation of air reduces the activity, particularly with the fast starter which virtually became slow under these conditions. There is thus a competition between the effect of aeration upon the metabolism of these starters and the effect of keeping the organisms uniformly distributed. The effect of aeration upon the activity of starter cultures is referred to in Babel's review, and the results in Table 2 are in agreement with previously reported observations. In this connexion it is interesting to note that Golding, McCorkle & Milich (3) found that whilst agitation during incubation resulted in increased activity, this effect was apparently associated with the creaming of the milk, for it did not occur with separated milk.

Confirmation of the effect of cream rising upon the activity of these slow starters has been obtained by storing the pasteurized milk after inoculation both at 30° C. and at 5° C., the milk being set up in separating funnels and the gravity skim being carefully drawn off, so that the cream and skim portions could be examined separately. In addition to carrying out this examination after 6 hr. at both temperatures, the cream and skim portions were drawn off after only $1\frac{1}{4}$ hr. at 30° C. (Table 3, column 1), and after sampling, incubation at 30° C. continued on these portions for the remainder of the normal 6 hr. period, i.e. for a further $4\frac{3}{4}$ hr. The further cream layer which had formed on the skim fraction was again drawn off and examination made not only of this cream and the corresponding skim fraction, but also of the cream which had been drawn off after $1\frac{1}{4}$ hr. at 30° C. (Table 3, column 4).

Table 3 shows that a considerable proportion of slow starter organisms is carried up with the rising cream and that this takes place at 5° C. as well as at 30° C. Multiplication can therefore be excluded as an explanation for the difference in counts. While the ratio between the number of organisms in the cream and in the skim milk is radically changed within $1\frac{1}{4}$ hr., column 4 shows that the tendency for the organisms to rise with the cream

persists for the next 4¾ hr. It may well be that the low count in the skim milk after 6 hr. at 30° C. (see column 2) is due to the continuous rising of the organisms, which may mask any multiplication taking place therein.

Table 3. *Distribution of slow starter organisms and acidity in gravity cream and skim milk when inoculated whole milk is stored at* 30° *and* 5° C.

(Plate counts: $\times 10^6$. Tryptone yeastrel lactose beef extract agar 3 days, 30° C.)
Initial count of inoculated whole milk: 3·4.

	(1) After 1¼ hr. at 30° C.		(2) After 6 hr. at 30° C.		(3) After 6 hr. at 5° C.		(4) After further 4¾ hr. at 30° C.		
								Skim	
	Cream	Skim	Cream	Skim	Cream	Skim	Cream	Cream	Skim
Butter fat (%)	19·5	2·33	27·4	1·65	15·4	2·10	—	—	—
Count $\times 10^6$	69	2	428	7·6	30	1·5	221	1015	5·9
Acidity (%)	0·150	0·135	0·425	0·185	0·125	0·135	0·590	0·470	0·180

Control: inoculated whole milk 6 hr. at 30° C., then shaken		Skim after further 4¾ hr. at 30° C., then shaken	
Plate count	45	Plate count	54
Acidity (%)	0·200	Acidity (%)	0·200

In Table 4, the rising of slow organisms with the cream is contrasted with the behaviour of a fast starter, which remains uniformly distributed throughout the cream and skim milk. If a whole milk is heated to a time/temperature combination resulting in a serious reduction of its creaming property, then the slow starter remains uniformly distributed throughout the cream and skim when stored at 5° C. and the rate of multiplication at 30° C. is of the same order in both.

Table 4. *Distribution of starter organisms found in gravity cream and skim when inoculated whole milk is stored for various times at* 5° *and* 30° C.

(Plate counts: $\times 10^6$.)

			Storage time (hr.)						
				1¼		4		6	
Starter	Heat treatment of milk	Storage temp. (° C.)	0 Mix	Cream	Skim	Cream	Skim	Cream	Skim
Slow	62·8° C./30 min.	5	8	48	3	55	4	58	2
Fast	62·8° C./30 min.	5	10	7	7	5	6	6	7
Slow	76° C./30 min.	5	11	10	7	9	7	10	8
Fast	76° C./30 min.	5	10	11	6	9	9	8	10
Slow	H.T.S.T.	30	6	52	4	159	3	746	32
Slow	H.T.S.T. + 76° C./30 min.	30	9	12	15	112	78	276	280

A number of starters was examined by this method, i.e. by inoculating H.T.S.T. pasteurized milk, cold storing it for a few hours in sterile separating funnels and plating out separately the gravity skim and cream. Under these conditions, whereas fast starters showed little difference in count between skim and cream, most of the slow starters showed a marked difference, the ratio of plate count in cream and skim being as high as 20:1 in some cases.

As creaming appeared to be a major factor in the activity of certain starters when tested in pasteurized milk, it was of interest to establish the rate of acid development in pas-

teurized and homogenized milks. Similar results were obtained, either with commercial pasteurized and homogenized milks or with raw and corresponding homogenized raw milk, both being subsequently laboratory pasteurized. Table 5 shows typical results obtained with a slow starter, together with those obtained on the same milks after re-heating to 82° C. (180° F.).

Table 5

	Acidity produced by a slow starter in			
			Re-heated to 82° C.	
Hr. at 30° C.	Pasteurized milk	Homogenized milk	Pasteurized milk	Homogenized milk
0	0·140	0·135	0·140	0·145
1	0·140	0·135	—	—
2	0·145	0·160	0·150	0·155
3	0·155	0·180	0·180	0·180
4	0·185	0·235	0·240	0·250
5	0·205	0·315	0·335	0·340
6	0·235	0·490	0·490	0·485

It will be seen that elimination of creaming, either by homogenization or by heating to 82° C. considerably enhanced the activity of the starter, and that heating homogenized milk to 82° C. did not bring about any increase in activity.

Effect of presence of rennet or agar on activity

During the course of this preliminary work on slow starters our attention was drawn to the stimulating effect of rennet upon the activity of certain slow starters. This effect has been confirmed and was found to apply to all starters which were affected by cream rising in the activity test. Fast starters, as judged by this test, are not affected to any extent by the presence of rennet. The results of an experiment are given in Table 6 and are typical of those obtained.

Table 6. *Acidity developed after* 6 *hr. at* 30° C.

	Starters used			
Milk	496 (slow)	760 (slow)	803 (slow)	852 (fast)
H.T.S.T. pasteurized	0·175	0·210	0·190	0·585
H.T.S.T. pasteurized + rennet	0·325	0·490	0·480	0·540
Homogenized	0·370	0·510	0·470	0·470
Homogenized + rennet	0·380	0·490	0·480	—

The concentration of rennet in the milk was the same as that commonly used in the cheese vat, namely 1 ml. per gallon, and was obtained by adding a 1/500 dilution of commercial rennet in Ringer solution to the inoculated milk (11 ml.). This addition was made immediately after the addition of the starter. Much difficulty was experienced in obtaining satisfactory agreement between replicate tubes owing to the firm nature of the rennet clot. Often this clot had contracted considerably by the end of the 6 hr. incubation, and it was not possible to disperse this sufficiently, either before or during titration, even though this was carried out in the tube in the presence of glass beads. For this reason it was decided to use agar instead of rennet, and the above difficulty was immediately overcome.

Table 7 gives comparative figures for the activity of three starters, one slow, one fast and one intermediate, in the presence of rennet and agar, the strength of the latter being 1 ml. of a 3% solution per 11 ml. inoculated milk. At this concentration the presence of agar results in a soft clot which is easily dispersed by shaking.

Table 7. *Acidity developed after 6 hr. at* 30° C.

	Starters used		
H.T.S.T. pasteurized milk plus	803 (slow)	865 (fast)	818 (intermediate)
Ringer (control)	0·245	0·610	0·405
Rennet	0·510	0·610	0·575
Agar	0·565	0·665	0·600

As will be seen from Table 7, the acidity developed in the presence of agar is usually somewhat higher than in the presence of rennet, but this is not felt to be a disadvantage. Apart from the probable inaccuracy of a single determination in the presence of rennet, the various acidity determinations normally made on the whey during cheese manufacture are but reflexions of acidity development in the curd and the use of a neutral gel such as agar will give a more accurate assessment of the potential activity of a cheese starter throughout manufacture than rennet, even though the latter is used in manufacture.

Since aeration tends to depress starter activity, the higher activity obtained in the presence of rennet and somewhat more so in the presence of agar is probably not only due to the prevention of creaming, but also to some degree of anaerobiosis developed in the milk gel during the 6 hr. incubation period.

The possible influence of 'agglutinin' on the activity of slow starters

'Agglutinin' is generally believed to be responsible for the natural creaming of milk, and experiments have been made to compare the activity of slow starters and the rate of creaming in milks heated to various temperatures using a Laboratory H.T.S.T. plant. The figures in Table 8 show that the activity markedly increases between 73° and 76° C. (163·5° and 168·5° F.), and that this increase corresponds to a marked drop in cream line, presumably due to the progressive inactivation of 'agglutinin' which occurs with rising temperatures.

Table 8. *Slow starter* (803)

Heating temp. (° C.)	Activity 6 hr. at 30° C.	Cream line (% by volume) 30 min.	45 min.	1 hr.	2 hr.	4½ hr.	6 hr.
69	0·200	7	7	7	6½	6	6
71·5	0·235	3	3¾	4	4¼	4	4
73	0·260	½	¾	2	2¾	3	3
76	0·370	Tr	⅓	½	½	¾	¾
79	0·455	Tr	⅓	½	½	¾	¾
82·5	0·470	Tr	⅓	½	½	¾	¾

By separating raw whole milk, heating the cream and separated portions to either 63° C. or 76° C. for 30 min., and determining the activity of a slow starter in whole milk reconstituted from these heated fractions, it has been found that the activity obtained depends upon the temperature to which the separated milk has been heated. It is not

affected by the temperature to which the cream has been heated. If the separated milk had been heated to 76° C./30 min. then the activity was always excellent (0·46–0·57), whereas if the separated milk had been laboratory pasteurized at 63° C. for 30 min., the activity was always poor (0·25–0·29). Results illustrating this are given in Table 9 and indicate that both the activity and the creaming properties were dependent only on the heat treatment of the separated milk.

Table 9

Heated 30 min. to ° C.			Cream line at 30° C. after		
Cream	Separated milk	Activity	30 min.	1 hr.	5 hr.
63	63	0·280	Nil	2	4
63	76	0·615	Nil	1	1·5
76	63	0·290	Nil	2	4
76	76	0·570	Nil	1	1·5
		Control whole milk			
	63	0·235	4	5	5
	76	0·450	1	1	2
		Control separated milk			
	63	0·390	—	—	—
	76	0·515	—	—	—

Temperature of separation, 46° C.

It has been claimed (5) that the temperature of separation influences the distribution of agglutinin between the cream and separated milk fractions. The experiment was therefore repeated with cream and separated milk prepared by cold separation (8° C. approx.).

Table 10

Heated 30 min. to ° C.			Cream line at 30° C. after		
Cream	Separated milk	Activity	30 min.	1 hr.	5 hr.
63	63	0·255	5·3	6	7·3
63	76	0·465	2·8	3·3	6·6
76	63	0·260	6·6	6·6	6·6
76	76	0·560	3·3	3·3	4·6

Temperature of separation 8° C.

These results are complicated by the fact that the rate of cream rising was in all cases greater than in Table 9, and this is presumed to be due to the formation of clusters of globules which were not dispersed under the conditions of test-tube pasteurization.

However, these results again show that the temperature of pasteurization of the separated milk fraction is the controlling factor in activity and rate of creaming.

Despite the good creaming properties illustrated in Table 10, it is reasonable to assume that the enhanced activity of slow starters in milks reconstituted from separated milk heated to 76° C. for 30 min., and cream heated to either 63° C. or 76° C., is due mainly to inactivation of agglutinin in the separated milk.

The lower activity obtained in separated milk heated to 63° C. for 30 min. as compared with the same milk heated to 76° C. for 30 min. (Table 9) is also believed to be due to agglutination, and the resultant tendency for these agglutinated organisms to deposit in the tube during the test.

This influence of 'agglutinin' on activity was confirmed by microscopic examinations described in the following section.

Microscopic examination of starters

In view of the effect of cream rising on the activity of some starters, microscopic examination of a number of starters was made to see whether there was any relationship between activity and chain length. No such relationship was detectable in commercial H.T.S.T. pasteurized milk and, in fact, one of the most active starters formed very long chains. In other words, slowness of those starters affected by cream rising is not due to length of chain. In milk heated to 76° C. for 30 min. it was noticeable that the slow starters grew in much shorter chains and that there was no tendency to clustering.

This question of clustering was then followed up by examining the growth of starters in whey. The whey was prepared by renneting H.T.S.T. or laboratory pasteurized milk and Seitz filtering prior to inoculation with starter. After about 4 hr. incubation at 30° C. in the case of slow starters a heavy flocculum commenced to form, which, when observed in a hanging drop, showed masses of agglutinated chains. No flocculum developed with these slow starters when they were grown in whey prepared from milk heated to 76° C. for 30 min., although growth was better as judged by opacity and low pH (B.C.P.). With fast starters, no flocculum formed unless the pH fell below 4·5, which sometimes occurred between $5\frac{1}{2}$ and 6 hr. at 30° C. In these cases the precipitate consisted of protein, and there was no indication of the presence of agglutinated organisms.

By incorporating a phosphate buffer in the whey, this precipitate could be prevented from forming during the 6 hr. incubation at 30° C. with fast starters, but its presence did not delay the formation of the flocculum when slow starters were grown in whey prepared from H.T.S.T. or laboratory pasteurized milk.

It has also been observed that slow starters, which give a better activity in separated and homogenized milk, tend to form a deposit on the bottom of the test-tube after 6 hr. at 30° C., whereas fast starters do not, when grown in either kind of milk. Examination of this deposit reveals enormous numbers of starter organisms, presumably due to agglutination.

II. KEEPING QUALITY OF HEAT-TREATED MILK

In his work on the keeping quality of heat-treated milks Galesloot (4) noted that milks pasteurized in the laboratory, using various time/temperature combinations, deteriorated at varying rates when stored at 20° C. or 27° C. Milk heated to 85° C. for 5 min. had a poorer keeping quality than milk heated to 63° C. for 35 min. More recently (6), Galesloot has examined the effect of post-pasteurization contamination on such laboratory pasteurized milks by inoculation of these milks with bacteria usually responsible for deterioration of commercial H.T.S.T. pasteurized milk. After storage overnight at 15° C. or 20° C. the milks were plated, and from the results Galesloot concluded that certain organisms such as lactic streptococci, flavobacteria and some species of *Pseudomonas* are inhibited in milk pasteurized at 63° C. for 35 min., whereas other *Pseudomonas* bacteria, *Achromobacter* and coliform organisms showed no such inhibition. The author suggested the presence of a heat labile inhibitor as probably responsible for the improved keeping quality of low-temperature pasteurized milk.

For many years we have been aware that the keeping quality of homogenized milk is generally slightly inferior to that of pasteurized milk—both pasteurized by the H.T.S.T. process 71·5° C. (161° F.) for 15 sec.—as judged by the acidity developed during storage

at 26° C. for 24 hr. Many explanations have been suggested, such as contamination from the homogenizer, breaking up of clumps of organisms, etc., but these have not been found satisfactory when critically examined.

The work just reported on the effect of cream rising upon the activity of certain starters suggested a more likely explanation for if, as suggested, certain starter organisms are agglutinated in low-temperature heated milk (> 71·5° C./15 sec.) and carried up by the cream rising on storage, then it would be reasonable to assume that this might well occur with other types of bacteria.

In investigating this possibility, the milks throughout were stored for 24 hr. at 26° C. in $6 \times \frac{5}{8}$ in. test-tubes, prior to measurement of the acidity developed.

Effect of agar

As with starters, a 3% agar solution was added at the rate of 1 ml./10 ml. of sample, and Table 11 shows some preliminary results.

Table 11

Milk	Acidity after 24 hr. at 26° C.	
	+ Ringer	+ 3% agar
1. H.T.S.T. pasteurized	0·175	0·185
2. H.T.S.T. pasteurized	0·165	0·200
3. H.T.S.T. pasteurized (Channel Islands)	0·180	0·295

Duplicate tubes containing B.C.P. showed that whilst acid development was uniform throughout the tube in the presence of agar, the cream layer in the control tubes showed acid development, particularly in the case of the Channel Islands milk. These results suggested that the pasteurized milks examined contained organisms affected by cream rising.

Effect of homogenization

Commercial samples of homogenized milk were taken immediately after homogenization, together with corresponding samples of pre-heated milk prior to homogenization. Both milks were laboratory pasteurized (71·5° C. 15/sec.), cooled and inoculated with varying amounts of raw milk and with coliform organisms. After 17 and 24 hr. at 26° C. the acidity developed was measured. Table 12 gives the results obtained, and these support the view that homogenization, by maintaining a uniform distribution of organisms throughout the milk, has reduced the keeping quality of the milk as judged by acidity developed after storage in tubes overnight.

Table 12

	Acidity after storage at 26° C.			
	17 hr.		24 hr.	
	Preheated	Homogenized	Preheated	Homogenized
Added to milk:				
Ringer solution	0·145	0·150	0·160	0·200
Dilution of *Bact. coli* culture	0·150	0·150	0·160	0·195
0·002% raw milk	0·155	0·180	0·170	0·430
0·02% raw milk	0·195	0·250	0·230	0·572
0·2% raw milk	0·250	0·355	0·285	0·735

Effect of various heat treatments upon keeping quality

Following Galesloot's work, experiments have been made using homogenized milk and the corresponding pre-heated milk, subsequently heated as follows: (*a*) Laboratory pasteurization 63° C. for 30 min.; (*b*) flash heating in flasks to 82° C.; (*c*) flash heating in flasks to 96° C.

To these milks were added (1) 0·2% raw milk, and (2) 0·1 ml. of 10^{-5} dilution of a 24 hr. broth culture of *Bact. coli* I per 10 ml. and results are given in Table 13.

Table 13

	Acidity after storage at 26° C. for 17 hr.			Acidity after storage at 26° C. for 24 hr.		
	63° C./ 30 min.	82° C. flash	96° C. flash	63° C./ 30 min.	82° C. flash	96° C. flash
Preheated milk containing:						
Ringer	0·155	0·140	0·145	0·175	0·140	0·140
Bact. coli	0·155	0·155	0·160	0·175	0·215	0·230
Raw milk	0·180	0·395	0·400	0·680	0·740	0·780
Homogenized milk containing:						
Ringer	0·195	0·155	0·150	0·290	0·190	0·195
Bact. coli	0·225	0·160	0·165	0·305	0·235	0·210
Raw milk	0·280	0·435	0·405	0·615	0·795	0·700

These results, taken in conjunction with those in Table 12, show that for a given post-pasteurization contamination, homogenized milk has a poorer keeping quality than the corresponding non-homogenized milk when these milks are laboratory pasteurized at 63° C. for 30 min. This difference is largely eliminated when both kinds of milk are heated to higher temperatures or, put in another way, post-pasteurization contamination in non-homogenized milk is more evident in the milks heated to high temperatures (82 and 96° C.).

These findings throw new light on Galesloot's results and suggest that the heat labile inhibitor is 'agglutinin'.

DISCUSSION

The creaming of milk has been reviewed by Dunkley & Sommer(5), and the general conclusions reached by these authors as a result of their own work on this subject, are confirmed by the results we have obtained both with starter activities and the keeping quality test for pasteurized and homogenized milk.

It seems clear that, when using laboratory tests involving storage of whole milk which has neither been homogenized nor heated to a temperature sufficiently high to inactivate the 'agglutinin', the assessment of bacterial metabolism by acidity development will be complicated by the rising of the fat globules to form a cream layer. Furthermore, the extent to which these tests are affected is primarily due to agglutination of bacteria, though the presence of rising clusters of fat globules is necessary to remove them from the body of the milk into the cream layer. The result of this depletion of the body of stored milk of its bacterial content, by agglutination and cream rising, is a lower acidity value than would have been obtained in the stored milk if either agglutination had not occurred or if the fat globules had been prevented from rising as in homogenized milk or in milk containing agar or rennet.

One cause of apparent slowness in starters is therefore due to the fact that certain starters are agglutinated in vat milk, assuming this has not been heated sufficiently to inactivate the 'agglutinin'. It is emphasized that such starters are not, as far as we are aware, slow in the vat, owing to the presence of rennet which prevents any cream rising. Thus this slowness is only apparent, due to the nature of the laboratory test used. It would be much more practical, therefore, to carry out such tests in vat milk containing agar to prevent cream rising. Some workers have preferred using autoclaved milk or even homogenized milk, but it is felt that the above modification is more logical. Similarly, in assessing the keeping quality of milk it seems clear that the same considerations apply, and it appears reasonable to conclude that the difference between the keeping quality of pasteurized and homogenized milk, which is often apparent from determination of acidity in storage tests, is mainly due to this combined effect of agglutination and cream rising.

No attempt has been made to study 'agglutinin' further, for our main concern has been to draw attention to a possible practical error in the assessment in the laboratory, both of starter activities and keeping quality of commercial pasteurized and homogenized milk.

SUMMARY

1. Criticism is directed towards the method of assessing cheese-starter activities, whereby pasteurized milk (vat milk) is inoculated with starter and its activity judged by the acidity developed after 6 hr. at 30° C.

2. It has been found that certain starters are slow when subjected to this laboratory test, owing to the fact that they are agglutinated in such milk and consequently carried up into the cream layer, thus producing a lower acidity in the milk.

3. Whilst such starters show higher activity in homogenized or autoclaved milk, it is suggested that starter activity tests should be carried out in the presence of agar in order to prevent cream rising and thus simulate conditions in the cheese vat. Agar is preferred to rennet for this purpose.

4. It is emphasized that such apparently slow starters are not slow when used in the cheese vat.

5. Evidence is given that keeping-quality tests on pasteurized and homogenized milks involving storage of the milk are also affected by the same factors, which may result in homogenized milk having a poorer keeping quality than pasteurized milk.

The authors wish to thank Mr L. J. Meanwell, who supplied the various starters examined, for his helpful comments and in particular for drawing our attention to the apparent stimulating action of rennet upon certain starters.

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669. STEAM DISTILLATION OF TAINTS FROM CREAM

III. FACTORS AFFECTING RATE OF REMOVAL OF THE REFERENCE SUBSTANCES, DIACETYL AND ACETOIN

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(With 6 Figures)

The use of diacetyl and acetoin as reference substances for investigations on the efficiency of cream-deodorizing equipment has been discussed in earlier papers (1,2,3). It has been shown (3) that in the Vacreator* vacuum-pasteurizer diacetyl is removed from cream somewhat more slowly than from water, per unit weight of steam condensed. It seemed desirable that this finding, made with factory equipment of pilot-plant scale, should be checked with laboratory apparatus permitting a more exact control of the conditions of steam distillation and so a more accurate estimate of the vapour/liquid equilibrium coefficient. The measurements were made in the continuous vaporization equilibrium still already described (4). A similar investigation was made for acetoin.

Diacetyl

Vapour/liquid equilibrium relationship for diacetyl in cream. Results for two runs with diacetyl in cream are shown in Fig. 1. The equilibrium coefficient, C_V/C_L (concentration-in-vapour/concentration-in-liquid at time of partition), was found to be uniform over the range of concentrations studied, and to be 26 for a cream containing 36 % fat and 28 for a cream containing 39 % fat. These results are lower than for diacetyl in water solution, for which the equilibrium coefficient has been shown to be 39·3.

The solubility butterfat/water partition coefficient for diacetyl is 0·74 at 180° F. (2). Extrapolation from the rate of rise in this coefficient between 100 and 180° F. indicates that at 212° F. it would be 0·80. The concentration C in the serum of a cream containing 36 % fat and 10 p.p.m. of diacetyl (on the assumption that lactose does not affect the partition coefficient) would be given by the equation

$$36 \times 0{\cdot}80C + 64C = 100 \times 10 \quad (C = 10{\cdot}8 \text{ p.p.m.})$$

If equilibrium of diacetyl between vapour and aqueous liquid is established as quickly with cream as with water, and if there is no significant delay in the re-establishment of the solubility partition equilibrium of diacetyl between the butterfat and serum in the cream, or if diacetyl passes directly from the fat globules into the vapour, the over-all equilibrium coefficient for diacetyl in cream should be higher than for diacetyl in water. Further investigations were therefore necessary to establish the cause of the low values recorded above for this coefficient with cream.

* Vacreator is the trade name of the Murray vacuum-pasteurizer manufactured by Murray Deodorizers Ltd., Auckland, New Zealand.

Effect of length of uptake-tube on vapour/liquid equilibrium coefficient for diacetyl in cream. If delay in re-establishment of solubility partition equilibrium of diacetyl between butterfat and water in the cream is the cause of the low values for the vapour/liquid equilibrium coefficient for diacetyl in cream, it would be expected that use of a longer uptake-tube in the laboratory apparatus, giving a longer contact of steam and cream, would result in a rise in the coefficient. Changing the length of the uptake-tube from 14 to 23 in., however, did not significantly alter the coefficient for a cream of 46% fat content.

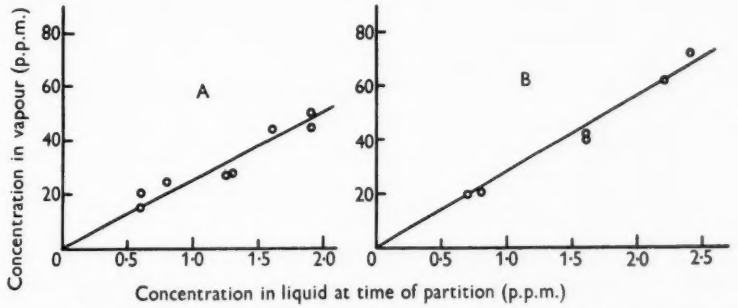

Fig. 1. Vapour/liquid equilibrium relationship for diacetyl in cream. A, 36% fat cream, C_V/C_L, read from line = 26. B, 39% fat cream, C_V/C_L, read from line = 28.

Comparison of equilibrium coefficients for diacetyl in water and in skim milk. The equilibrium coefficients found for diacetyl in skim milk and in some dilutions of skim milk with water, given in Table 1, show that skim milk depresses the vapour/liquid equilibrium coefficient of diacetyl in dilute solutions. The low values for the coefficient for cream, therefore, may be attributed, in part at any rate, to this depressing effect of some constituent in the cream serum.

Table 1. *Vapour/liquid equilibrium coefficients for diacetyl in skim milk and in diluted skim milks*

Description of liquid at time of partition	Vapour/liquid equilibrium coefficient
Skim milk undiluted	28·4
Skim milk containing 38% added water	31·0
Skim milk containing 91% added water	39·2
Water	40·0

Effect of lactose on the vapour/liquid equilibrium coefficient for diacetyl in water. The results,* given in Fig. 2, show that with increase in concentration of lactose in dilute aqueous solutions of diacetyl there is a progressive decrease in the vapour/liquid equilibrium coefficient. The coefficient for a 5% solution of lactose (which is approximately equivalent to the concentration in skim milk or in cream serum), read from the curve in Fig. 2, is 30, a figure which is in reasonable† agreement with the value of 28·4 found for skim milk (Table 1).

* Lactose was found not to affect the estimation of diacetyl by the method of Pien, Baisse & Martin (5).

† Apart from any vagaries of the laboratory continuous vaporization equilibrium still the results for the equilibrium coefficient can vary considerably because of experimental error in the colorimetric estimation of diacetyl.

It was shown that in a cream of 36 % fat content and containing 10 p.p.m. of diacetyl the concentration in the cream serum would be 10·8 p.p.m. If it be assumed that in the laboratory vapour/liquid equilibrium apparatus the vapour is in equilibrium with the serum and that as diacetyl is removed from the cream the solubility partition equilibrium establishes itself instantaneously, the equilibrium coefficient for cream, computed from the value 28·4 for skim milk, would be $10{\cdot}8/10{\cdot}0 \times 28{\cdot}4 = 30{\cdot}7$, somewhat higher than the value of 26·0 found for cream of this fat content (see above). The results show that in

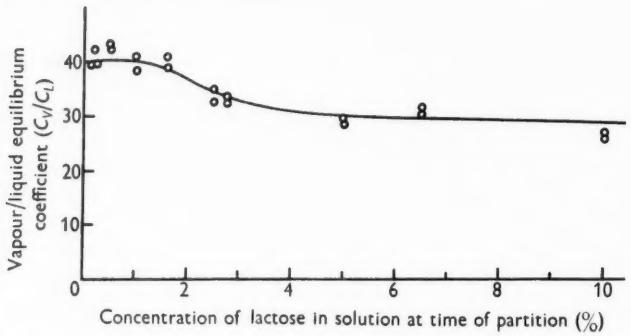

Fig. 2. Effect of concentration of lactose on the vapour/liquid equilibrium coefficient for diacetyl.

equipment giving concurrent flow of steam and cream there is a fairly close approach to equilibrium of diacetyl as between the vapour and the liquid, but there are indications that the presence of fat causes some retardation in the attainment of equilibrium. If this latter factor is operating, the effect should be greater with substances showing a higher relative solubility in the fat, and also with substances of higher molecular weight, which could be expected to diffuse more slowly from the fat to the aqueous phase for re-establishment of the solubility partition equilibrium.

Influence of diacetyl concentration on the depressing effect of lactose on the vapour/liquid equilibrium coefficient for diacetyl. Readings were taken with the continuous vapour/liquid equilibrium apparatus with solutions of diacetyl containing 10 % lactose and varying concentrations of diacetyl. The lactose content of the solution was lowered somewhat by condensation of steam in the vapour/liquid mixing device—at time of partition it was of the order of 9·0 %. The results for the vapour/liquid equilibrium coefficient are shown in Fig. 3 plotted against concentration of diacetyl in the liquid at time of partition. It is clear that the depressing effect of lactose on the equilibrium coefficient was uniform over the range of diacetyl concentration studied, viz. from 1 to 65 p.p.m. in the liquid at time of partition.

Effect of salt on vapour/liquid equilibrium coefficient for diacetyl in water. Salt has been used (3) in cream-deodorization investigations for checking the dilution that may occur in processing. At the low concentrations employed (less than 0·5 %), and within the range of accuracy of the trials using commercial equipment, there was no effect of salt on the rate of removal of diacetyl from water. In Fig. 4 results are given of an investigation of the effect of a range of concentrations of salt in water on the vapour/liquid equilibrium coefficient for diacetyl. At low concentrations, up to 1 % of salt in the water, there was no significant effect, in agreement with the findings with commercial equipment. But with

higher concentrations of salt there was a very considerable increase in the equilibrium coefficient, corresponding to the normal expected 'salting-out' effect.

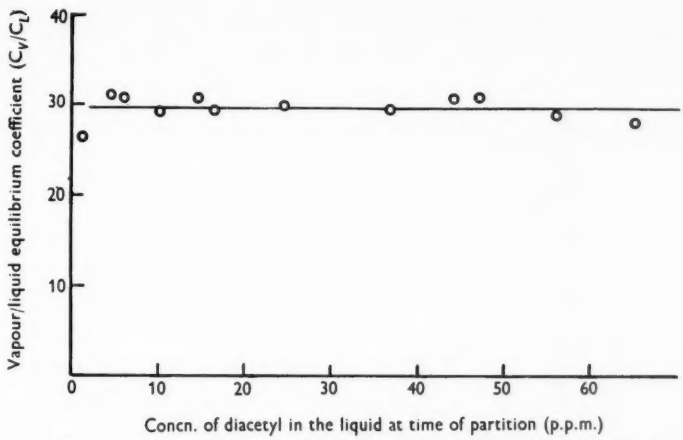

Fig. 3. Effect of concentration of diacetyl in the liquid on the vapour/liquid equilibrium coefficient for diacetyl in 9% aqueous solution of lactose.

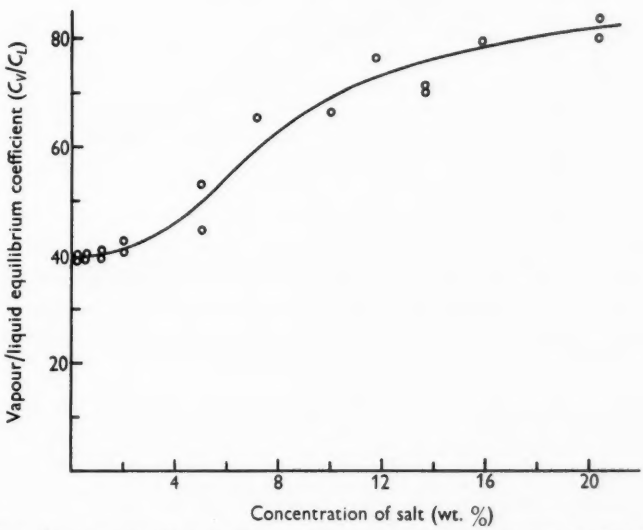

Fig. 4. Effect of sodium chloride in solution on the vapour/liquid equilibrium coefficient for diacetyl.

Acetoin

Equilibrium coefficient for acetoin in cream. The results given in Fig. 5 for constant-volume distillation of a 10 p.p.m. solution of acetoin in cream containing 50% fat show that the rate of removal from cream is considerably more rapid than from water. It was found (2) that the butterfat/water solubility partition coefficient for acetoin is approximately 0·20 and that it is not significantly affected by temperature. In a 50% cream

containing 10 p.p.m. of acetoin the concentration of acetoin in the serum would be given by the equation

$$50 \times 0{\cdot}2C + 50C = 10 \times 100 \quad (C = 16{\cdot}7 \text{ p.p.m.}).$$

The concentration of acetoin in the first 10 ml. distilled from the cream was 24·6 p.p.m., and the concentration in the corresponding first 10 ml. distilled from water was 14·0. On

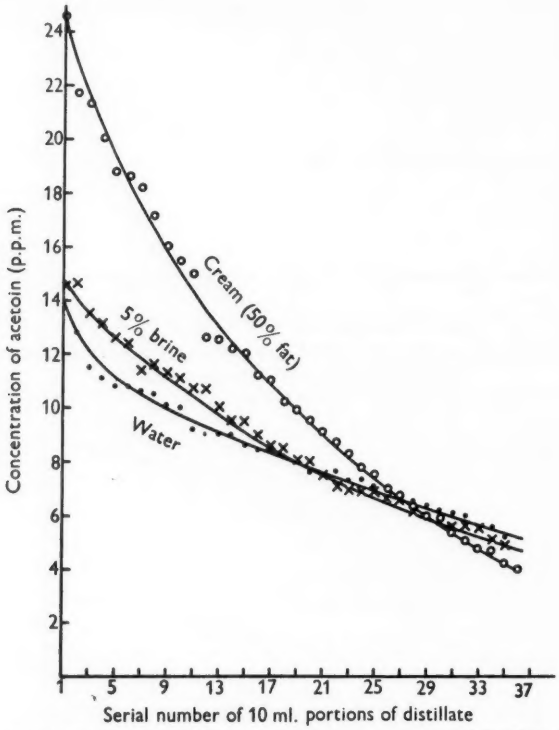

Fig. 5. Changes in concentration of acetoin in successive 10 ml. portions of distillate from a 10 p.p.m. concentration of acetoin in 500 ml. water, in 5% brine and in 50% cream.

the assumption that the initial concentration in the cream serum was, as computed above, 16·7 p.p.m., and without allowing for an effect of lactose on the equilibrium coefficient, it would be expected that the concentration of acetoin in the first 10 ml. distilled from cream would be $16{\cdot}7/10 \times 14{\cdot}0 = 23{\cdot}4$ p.p.m., which is in close agreement with the concentration found, 24·6 p.p.m. It appears, therefore, that lactose has no appreciable effect on the equilibrium coefficient for acetoin. This is confirmed by the results in Table 2, obtained with the laboratory continuous vaporization equilibrium apparatus.

Effect of salt on vapour/liquid coefficient of acetoin in water. Trials on commercial cream-deodorization equipment (3) had not indicated any appreciable influence of low concentrations of salt on the rate of removal of acetoin with steam. The results obtained with the laboratory apparatus, and given in Fig. 6, confirm this finding for low concentrations of salt, but show that with increase in concentration of salt there is a progressive increase in the vapour/liquid equilibrium coefficient.

Table 2. *Vapour/liquid equilibrium coefficients of acetoin in water containing a range of concentrations of lactose*

Concentration of lactose in liquid at time of partition	0	0·9	2·5	4·7	6·8	9·1
Vapour/liquid equilibrium coefficient (C_V/C_L)*	1·29	1·26	1·33	1·31	1·43	1·31

* Acetoin was determined by oxidation to diacetyl and estimation by the method of Pien, Baisse & Martin (5).

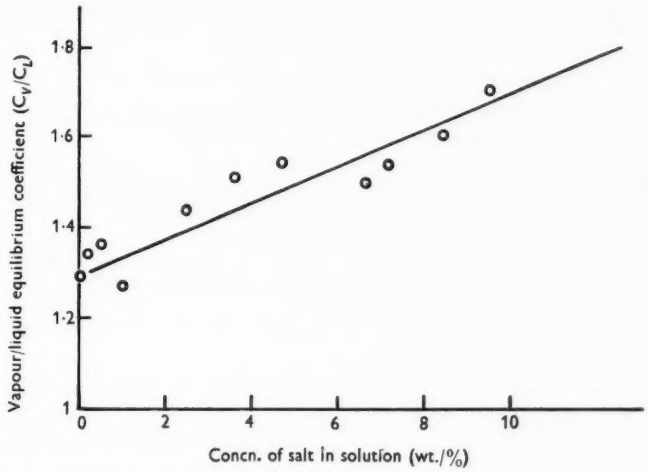

Fig. 6. Effect of sodium chloride in solution on the vapour/liquid equilibrium coefficient of acetoin in water at concentrations of 5–10 p.p.m.

DISCUSSION

The finding that lactose has such a marked effect on the proportion of diacetyl passing from the cream into the steam is of particular interest, because it introduces the possibility that it may have a similar and possibly more pronounced effect with objectionable tainting substances in cream.

SUMMARY

1. The vapour/liquid equilibrium coefficient for *diacetyl* in 35–40% cream has been found to be of the order of 26–28, compared with 39·3 for water. In a 5% aqueous solution of lactose, equivalent to that present in milk and in cream serum, the coefficient was 30. The depression of the equilibrium coefficient by lactose in solution was shown to be independent of the concentration of diacetyl present in the liquid at time of partition, over the range 1–65 p.p.m.

2. The slower rate of removal of diacetyl from cream, per unit weight of steam condensed, as compared with its removal from water, was observed to be due mainly to this depressing effect of lactose on the equilibrium coefficient; but there was some evidence that delay in re-establishment of the solubility partition equilibrium as between butterfat and cream serum also contributed to the lowering of the equilibrium coefficient for diacetyl in cream as measured in the laboratory continuous vaporization equilibrium still.

3. Sodium chloride in the solution caused a rise in the equilibrium coefficient for diacetyl from the value of 39·3 for water to about 69 for a 10% salt solution and 82 for a 20% salt solution.

4. Lactose had no effect on the equilibrium coefficient for *acetoin*, but salt caused a rise from 1·29 for water to approximately 1·75 for a 10% salt solution.

5. The possible effect of lactose on the effectiveness of steam in cream deodorization equipment is referred to.

The author wishes to thank Mr E. R. Elley for valuable assistance with the experimental work.

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670. FURTHER STUDIES OF THE EFFECT OF PROCESSING ON SOME VITAMINS OF THE B COMPLEX IN MILK

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This work is a continuation of our earlier studies on the effects of commercial processing methods, as used in Britain, upon the nutritive value of milk(1–13). It had two main objectives: one was to add to the existing knowledge by including measurements of factors which were either unknown at the time of the earlier investigations or for which satisfactory methods of measurement other than biological have only recently been evolved; the other was to study the effects of new methods and recent modifications of existing ones.

Of late the general trend in milk processing has been towards the application of increasingly higher temperatures for short periods of time in various pre-treatments and also towards the use of high temperature in the main treatments themselves. In view of the heat lability of many nutritionally important constituents of milk, the nutritive effects of these new methods obviously demanded study.

The paper deals with seven vitamins of the B complex in seven heat-treated milk products made from one bulk of raw milk.

METHODS

Bulk milk. A bulk of 170 gal. of whole milk predominantly from Shorthorn and Friesian cows was obtained from a commercial dairy within 20 miles of the Dairy Department of Reading University. There it was emptied into an insulated stainless steel tank fitted with a mechanical agitator, and stored overnight at about 5° C. After thorough mixing, samples were withdrawn for processing when the milk was between 24 and 36 hr. old. With the exception of the ultra-high temperature (U.H.T.) sterilization, which was done at the National Institute for Research in Dairying, all treatments were done at the Dairy Department of Reading University on a commercial or pilot-plant scale.

Pasteurized milk (H.T.S.T. process). Eighty gallons of milk were pasteurized at 72° C. (holding for 15 sec.) in a commercial A.P.V. plate-type heat exchanger and the milk was sampled after having been cooled to about 5° C.

Sterilized milk (*in-bottle process*). Four gallons of milk were brought within 10 min. to 71·1° C. and then homogenized at a pressure of 2500 lb./in.^{2} and dispensed into pint bottles which were sealed immediately with 'Crown' corks of the type normally used. The bottles were heated in a steam autoclave for 30 min., some at 107·2° C. and others at 111° C.

Sterilized milk (U.H.T. *process*). Fifty gallons of milk were brought within 18 sec. to 85° C., homogenized in a two-stage homogenizer at 500 and 2000 lb./in.^{2} within about 23 sec. and sterilized by the ultra-high temperature process in an A.P.V. 200 gal./hr. HX plate-type stainless steel commercial plant. The milk was heated in it within 18·5 sec. to 135° C. and held at this temperature for about 2 sec. before cooling to 20° C. One gallon of this processed milk was dispensed into pint bottles, which were then sealed with 'Crown' corks and heated to 107·2° C. for 20 min. The second sterilization was necessary to simulate commercial conditions in which there is still no satisfactory method for aseptic bottling.

Evaporated milk. Fifteen gallons of milk, preheated at 95° C. for 10 min., were evaporated in a small stainless steel vacuum pan at a temperature between 52° and 54° C. to a specific gravity of 1·072 at 49° C. (33·9% milk solids). The milk reached this concentration in about 45 min.; 5·5 g. of sodium citrate were then added and the milk was homogenized at 2000 lb./in.^{2}. The citrate-stabilized, homogenized milk was then filled into standard (2-pint equivalent) cans and sterilized in a steam autoclave at 115° C. for 15 min. The cans were cooled in water immediately on removal from the autoclave.

Sweetened condensed milk. To 15 gal. of milk were added 25½ lb. of cane sugar and the mixture was preheated at 79·5° C. for 15 min. The evaporation was carried out in the same pan and under the same conditions as for the evaporated milk, but the striking point was 1·282 specific gravity at 49° C. (77·2% total solids including 33·8% milk solids). The condensed milk was then cooled to about 32° C. in a tubular condensed milk cooler made with stainless steel tubes and gun-metal end caps and, after standing for about 15 min., was further cooled to 24° C. before being filled into condensed milk cans of a standard size, 1¾ pints equivalent.

Roller-dried milk. Two gallons of milk were heated to 71·1° C. within 10 min. and then dried in a pilot-plant roller drier on twin rollers 8 in. long and 8 in. in diameter heated by steam at 60 lb./in.^{2}.

Spray-dried milk. Three gallons of milk were heated to 87·8° C. and then evaporated under reduced pressure in a glass apparatus at 45° C. in about 45 min. to about 40% milk solids. The concentrated milk, divided into two equal batches, was then spray-dried, in a Niro pilot plant in which milk comes in contact only with stainless steel and which uses a high-speed rotating wheel for atomization. Two different chamber temperatures were used: (*a*) 'low temperature'—inlet 150° C., outlet 75° C.; and (*b*) 'high temperature'—inlet 180° C., outlet 80–90° C. Each batch took 1 hr. to collect. The milk powder was on the average in the hopper half this time at 75° or 90° C. The collecting times were considerably longer than those under commercial conditions.

Sampling. After preparation, the several products were placed in a cold room at 3° C. Samples of each were then taken for the various determinations. The samples for microbiological tests were stored at −30° C. until required. The physico-chemical tests were completed within 48 hr. of sampling, and the samples were not frozen.

Methods of vitamin assay

The following vitamins were measured: thiamine, riboflavin, nicotinic acid, pantothenic acid, biotin, vitamin B_6 and vitamin B_{12}. In general, published procedures were used, which are briefly outlined below. Ford, Gregory, Porter & Thompson (14) and Gregory (15) give fuller details.

Chemical methods

Preparation of samples. Products that had been concentrated or dried were reconstituted to the same total milk solids content as the original milk. Sampling for assay was by means of pipettes calibrated for weight delivery of each particular product.

Thiamine was measured fluorimetrically by the thiochrome method of Jansen (16) in a Cohen-type fluorimeter (17), as described by Houston, Kon & Thompson (18). In addition, recovery tests were done with crystalline thiamine added just before the stage of oxidation to thiochrome.

Riboflavin. The methanol method of Emmerie (19) was applied with the omission of the potassium permanganate–hydrogen peroxide step which we have found unnecessary with cow's milk and its products. The fluorescence was measured in the same fluorimeter as for thiochrome, but a yellow filter (Wratten No. 12) was placed between the fluorescent substance and the photocell. The fluorescence was compared with that of a solution of known strength of crystalline riboflavin in 50% aqueous methanol.

Microbiological methods

Nicotinic acid. Weighed samples were heated with 0·2 N-H_2SO_4 for 20 min. in steam at 20 lb./in.^{2} pressure. The extracts were then brought to pH 4·8, diluted with water to known volume, and filtered. After adjusting to pH 6·8 the filtrates were assayed with *Lactobacillus arabinosus* 17–5 in the medium of Roberts & Snell (20), modified by omitting nicotinic acid and including riboflavin.

Biotin. The samples were prepared for test as for nicotinic acid. The same test micro-organism was used, and the same growth medium, modified by omitting biotin and including riboflavin.

Pantothenic acid. Weighed samples were dissolved in water and heated in flowing steam for 20 min. The solutions were cooled, made to pH 4·8 at known volume and filtered. The filtrates were made to pH 6·8 and assayed with *Lb. arabinosus* 17–5 in the medium of Roberts & Snell (20), modified by omitting pantothenic acid and including riboflavin.

Vitamin B_6. Weighed samples were autoclaved with 0·055 N-H_2SO_4 for 5 hr. at 20 lb./in.^{2} steam pressure, as recommended by Rabinowitz & Snell (21). The extracts were then adjusted to pH 4·8 at known volume, filtered and assayed with *Saccharomyces carlsbergensis* 4228 by the method of Atkin, Schultz, Williams & Frey (22) with the following modifications. The medium was supplemented with nicotinic acid and ammonium phosphate, as recommended by Hopkins & Pennington (23); the final liquid content of assay tubes was 5 ml.; the filled tubes were heated in flowing steam for 15 min., cooled in ice before inoculation and brought to 30° C. in a water-bath before transfer to a shaker in an air incubator.

Vitamin B_{12}. Extracts were prepared as recommended by the Analytical Methods Committee, Society for Analytical Chemistry (24), and assayed with *Lb. leichmannii* ATCC 4797 by the method of Skeggs, Nepple, Valentik, Huff & Wright (25), modified as described by Coates, Ford, Harrison, Kon & Porter (26). Some of the results were checked by assays with *Ochromonas malhamensis* (cf. Ford (27); Coates & Ford (28)).

Table 1. *Effect of different processing treatments on some of the vitamins of the B complex in milk*

Product				Vitamin content											
				Thiamine		Riboflavin		Pantothenic acid		Nicotinic acid		Vitamin B_6		Biotin		Vitamin B_{12}	
Description of milk	Total milk solids (g./100 g.)	Fat content (g./100 g.)	Concentration factor (calculated from fat content)	μg./g.	Loss on processing (%)	μg./g.	Loss on processing (%)	μg./g.	Loss on processing (%)	μg./g.	Loss on processing (%)	μg./g.	Loss on processing (%)	μg./g.	Loss on processing (%)	mμg./g.	Loss on processing (%)
Raw	12·02	3·39	—	0·46	—	1·6	—	2·6	—	0·72	—	0·22	—	0·019	< 10	3·2	—
Pasteurized (H.T.S.T.)	12·02	3·36	—	0·44	< 10	1·6	0	2·6	<10	0·71	0	0·20	<10	0·018	<10	3·0	<10
Sterilized																	
In-bottle process																	
(*a*) Before sterilization	12·09	3·39	—	0·41	<10	1·6	0	2·6	<10	0·72	0	0·22	0	0·019	<10	2·9	<10
(*b*) Sterilized at 107·6° C.	12·11	3·40	—	0·30	30–40	1·5	<10	2·5	<10	0·75	0	0·22	0	0·018	<10	<0·06	90–100
(*c*) Sterilized at 111° C.	12·07	3·39	—	0·25	40–50	1·5	<10	2·5	<10	0·75	0	0·22	0	0·018	<10	<0·06	90–100
Ultra-high temperature process																	
(*a*) U.H.T. alone	12·07	3·39	—	0·44	<10	1·5	<10	2·6	<10	0·73	0	0·23	0	0·017	<10	2·7	15–20
(*b*) U.H.T. then in-bottle sterilization	11·99	3·39	—	0·25	40–50	1·5	<10	2·3	10–15	0·80	0	0·22	0	0·019	<10	<0·06	90–100
Evaporated																	
(*a*) Before sterilization	33·90	9·58	2·83	1·2	<10	4·8	0	6·7	<10	2·0	0	0·57	<10	0·050	<10	2·9	70
(*b*) After sterilization	33·84	9·57	2·83	0·82	30–40	4·8	0	7·3	<10	2·0	0	0·61	0	0·047	10–15	1·0	90
Sweetened condensed																	
(*a*) Preheated	10·57	3·00	0·88	0·40	<10	1·3	0	2·4	<10	0·64	0	0·17	<10	0·015	<10	1·9	30
(*b*) After condensation	33·80	9·56	2·81	1·0	10–20	4·0	<10	7·5	<10	2·3	0	0·52	10–15	0·047	10–15	5·4	40
Roller-dried	97·75	28·4	8·36	2·7	20–30	11	10–15	23	<10	6·0	0	1·8	0	0·14	10–15	21	20
Spray-dried																	
(*a*) Low temperature	97·43	27·5	8·12	3·7	<10	12	<10	24	<10	6·1	0	1·8	0	0·13	10–15	17	35
(*b*) High temperature	95·54	28·1	8·28	3·3	10–15	12	<10	25	<10	6·1	0	1·5	0	0·13	10–15	16	35

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RESULTS

The results are summarized in Table 1. They show that with five of the vitamins—riboflavin, pantothenic acid, nicotinic acid, biotin and vitamin B_6—there was little or no apparent loss during processing.

Thiamine. As was expected (cf. Kon (29)) severe heat treatment resulted in considerable loss of thiamine: a loss of $< 10\%$ during H.T.S.T. pasteurization or spray drying contrasts with losses of 30–40% and 40–50% respectively during in-can sterilization of evaporated milk, and in-bottle sterilization.

Sterilization by the U.H.T. process caused little or no loss. This finding supports that of Bernhard, Gschaedler & Sarasin (30) that 'Uperization' of milk involves relatively little loss of thiamine. 'Uperization' denotes ultra-high temperature short-time pasteurization, and in some essentials resembles the U.H.T. process.

Vitamin B_{12}. Vitamin B_{12} was almost completely destroyed during in-bottle and in-can sterilization. The pre-sterilization treatment of the evaporated milk also caused considerable loss. The preparation of roller- and spray-dried milk entailed about 30% loss, and of sweetened condensed milk about 40%. The first sterilization by the U.H.T. process caused about 15% loss, compared with $< 10\%$ on H.T.S.T. pasteurization.

The values in Table 1 for vitamin B_{12} content were obtained by assay with *Lb. leichmannii*. Assays of several of the products with the protozoan *Ochromonas malhamensis* gave virtually identical results.

Table 2. *Vitamin B_{12} content of proprietary milk products* (mμg./g.)

Product	Vitamin B_{12}
Evaporated full cream, brand A	1·9
Evaporated full cream, brand B	1·0
Evaporated full cream, brand C	1·6
Sweetened condensed full cream, brand D	6·3
Sweetened condensed full cream, brand E	3·0
Sterilized (in-bottle)	0
'National' dried full cream*	22·3 (13–28)

* Six samples, representing different manufacturers' products, supplied for test by Medical Officers of Health at Grimsby, Cardiff, Plymouth, Maidstone, Bradford and Glasgow.

For purposes of comparison, Table 2 shows the vitamin B_{12} content of several commercial and proprietary milk products which, with the exception of the 'National' dried milk, were purchased at local food stores. The values can only be related to a conjectural value for fresh bulk milk, which is likely to approximate fairly closely to 4 mμg./ml.

DISCUSSION

Cow's milk is richer than human milk in vitamins B_6 and B_{12}, and in biotin and pantothenic acid (cf. Gregory (15)). The levels of riboflavin and nicotinic acid are somewhat lower, but on the whole cow's milk has been supposed to provide adequately for the needs of the human infant for these vitamins.

There is, on the other hand, increasing evidence for subtle differences in the chemical forms and associations of certain of the vitamins between milks from different species of animal (cf. for riboflavin, Anonymous (31); for vitamin B_{12}, Gregory & Holdsworth (32), Coates, Gregory, Harrison, Henry, Holdsworth & Kon (33), Gregory, Ford & Kon (34)). Until the metabolism of such vitamin complexes has been more fully investigated it

is perhaps early to assume that they are nutritionally equivalent for the different species.

It may, however, be argued that cow's milk, suitably modified, has been used for many years with apparent success in infant feeding. A more immediate consideration is that of the nutritional implications of the growing use of sterilized liquid milk; for here, in the loss of vitamin B_{12} and the reported inactivation of vitamin B_6, there may well be cause for concern.

Several workers have reported that the biological availability of vitamin B_6 in milk is impaired during the process of heat sterilization (cf. György (35); Tomarelli, Spence & Bernhart (36); Hassinen, Durbin & Bernhart (37)), and in general it appears that the microbiological tests, in particular those made with *Sacch. carlsbergensis* (Hodson (38)), tend to underestimate the extent of this loss on heating. It thus seems that our evidence for the heat stability of vitamin B_6 in milk may be largely illusory, and requires the confirmation of biological tests. These we are now carrying out, together with more refined studies by the methods of chromatography and ionophoresis. There is some inconsistency between different workers' estimates of the heat stability of vitamin B_6 in milk as judged by microbiological methods. It may in part reflect a wide variation in commercial practice in the sterilization of liquid milk in which temperatures from 110° to 121·1° C. are used for periods of from 5 to 30 min. We found that in-bottle sterilization for 120 min. at 115·5° C. caused an apparent loss of 49%, whereas at 110° C. for 30 min. the loss, when measurable, did not exceed 20%. But, doubtless, other factors than the actual process of sterilization are relevant to the reported loss of vitamin B_6 from sterilized and evaporated milk—for example, the prehistory of the milk in relation to aeration and exposure to light; oxygen in the head space; and the period and temperature of storage after sterilization.

Two features of special practical importance emerge from this work: (*a*) that during in-can or in-bottle sterilization serious loss of vitamin B_{12} takes place and, since these sterile milk products have many advantages for infant feeding, this loss may be of nutritional importance; (*b*) that in the recently introduced ultra-high temperature method milk is sterilized without loss of thiamine and with a loss of only about 15% of vitamin B_{12}, but the problem of aseptic bottling remains to be solved before the advantages of this method can be exploited.

The losses of thiamine reported in this paper indicate that some new methods introduced in the last 10 years have increased the adverse effect of processing on the nutritive value.

Further work which is in progress on the factors influencing the heat destruction of vitamin B_6 and vitamin B_{12} will be reported elsewhere.

SUMMARY

1. Pasteurized, sterilized, U.H.T. treated, U.H.T. treated–sterilized, evaporated, sweetened condensed, roller- and spray-dried milks were prepared simultaneously from one bulk of raw whole milk.

2. The preparation of any of these products entailed little or no apparent loss of riboflavin, pantothenic acid, nicotinic acid, vitamin B_6 or biotin.

3. Thiamine was destroyed in amounts ranging from less than 10% to 40–50%, increasing with the severity of the heat treatment.

4. Prolonged high temperature used in the sterilization of evaporated milk and of in-bottle sterilized milk caused nearly complete loss of vitamin B_{12}.

5. The new process of sterilization by the U.H.T. treatment alone when it was not followed by the, so far necessary, in-bottle sterilization caused little more loss of the vitamins measured than did H.T.S.T. pasteurization.

The authors are grateful to Mrs O. M. Bullock and Mrs P. J. Plack for technical assistance.

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671. THE ASSOCIATION BETWEEN THE FAT PERCENTAGE OF COWS' MILK AND THE SIZE AND NUMBER OF THE FAT GLOBULES

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Variations in the percentage of fat in the milk yielded by cows are sometimes accompanied by changes in the size of the fat globules and occasionally by changes in the number of the globules. Collier (1) took successive pints from one milking, and found a fairly steady increase in both the relative size and number of fat globules from the first to the last pint and showed that these increases accompanied the rise in the percentage of fat. Woll (2) found that when morning milk had a higher percentage of fat than evening milk the greater fat percentage was accompanied by an increase in the size of the fat globules, but that the number was the same for a given volume. Observations by King on the milk of diseased cows which developed elevated body temperatures showed that a rise in body temperature was accompanied by a fall in yield and by increases in the percentage of fat and the average diameter of the fat globules (3), but that there was no significant difference in the fat globule number per cu.mm. (4). Turner & Haskell (5) found that the diameter of the globule of average volume was 4·01μ for Jerseys, 3·27μ for Ayrshires and 3·12μ for Holsteins. Since the average fat percentage of Jersey milk is higher than that of Ayrshire milk, and since this breed's milk has a higher average fat percentage than Friesian milk, the fat globule diameter appears to vary with the fat percentage between breeds.

The object of the work described in the present paper was to determine whether different fat percentages in cows of the same breed were accompanied by variations in the fat globule diameter and number per cu.mm.

EXPERIMENTAL

The fifty-six Ayrshire and forty Friesian cows from which milk samples were taken were kept at the University of Liverpool Veterinary Field Station, and on five farms all situated within four miles of the Field Station. Care was taken to see that factors known to affect the size and number of fat globules did not have any influence on the samples examined. The greatest single factor affecting the size of the fat globules within a breed is advance in lactation. Eckles & Shaw (6) showed that the size declined very sharply during the first 6 weeks, then remained relatively constant for 5–6 months, and then declined much more rapidly towards the end of lactation. Woll (7) showed that there was an increase in the number of globules which accounted for the raised fat percentage as the lactation progressed. To avoid these trends samples were only collected between the 45th and 165th days after calving. Changes in a cow's environmental state appear to increase fat globule size. Gutzeit (8) found a distinct increase in the size of the globules when turning cattle on pasture in the spring or on housing them in the autumn, and Weigmann (9) found that in general the change from pasture to stall feeding caused an increase in the size of the

globules. To eliminate, as far as possible, the effect of sudden changes of environmental conditions and diet, all the samples were collected between 17 April and 30 May 1956, after the cows had been turned out day and night for at least 14 days with the grass providing for maintenance and the production of 3 gal. of milk in two of the Ayrshire and one of the Friesian herds, and for maintenance and 4 gal. production in the other three. Balanced rations consisting of home-grown cereals and proprietary brands of concentrates were fed for yields above those shown. The milk was thoroughly mixed before being sampled.

The figures for milk yield are the totals and the figures for fat percentage and fat globule size and number are the averages for consecutive morning and evening milkings, on one occasion for each cow, in the five herds which were milked twice a day, and for consecutive morning, afternoon and evening milkings, on one occasion per cow, in the other herd where three-times-a-day milking was employed. The samples of milk were thoroughly mixed before testing by rotating the bottles, but they were not agitated sufficiently to break the globules. The fat percentages were determined by the B.S.S. no. 696 (1936) Gerber method. For the estimation of globule size 0·5 ml. milk was placed in a watch-glass and to it was added 1 ml. of a glycerin-cochineal stain mixture as prepared by Turner & Haskell (5). A vaseline ring was made on a slide and, after thorough mixing, several drops of the stained milk were placed in it, and a cover-slip pressed down. The slides were then left in a flat position for an hour or more to allow the fat globules to rise. They were then examined with an oil-immersion lens and a graduated micrometer eyepiece and the diameters of a 100 globules on each slide were measured and the average diameter calculated. The dimensions of the eye-piece graduations were determined by the use of a stage micrometer as described by Mackie & McCartney (10). For counting the fat globules 1 ml. of milk was added to 9 ml. of water, and 1 ml. of this mixture was added to 9 ml. of a mixture of equal quantities of glycerine and water. A drop of this was placed on a Thoma-Zeiss haemocytometer slide, which was placed upside down for at least 20 min. so that the globules rose and came in contact with the ruled scale. The globules in three large squares and two small ones (giving a total of 50 small squares) were counted, and the number of globules per cu.mm. calculated.

RESULTS

The findings obtained in respect of the two breeds, Ayrshires and Friesians, have been summarized in Table 1. A fat percentage interval of 1 % has been chosen for convenience.

Table 1. *Details of the average fat globule diameter and number per cu.mm. of the cows grouped according to the fat percentage of their milk*

	Ayrshires			Friesians		
Fat percentage	No. of cows sampled	Fat globule diameter (μ)	Fat globule number per cu.mm.	No. of cows sampled	Fat globule diameter (μ)	Fat globule number per cu.mm.
2·6–3·59	18	3·012	3478	20	3·002	2761
3·6–3·59	24	3·237	3246	20	3·125	3071
4·6–5·59	14	3·383	3451	—	—	—

Within the rather limited range of this summary it will be observed that as the percentage of fat increased there was a corresponding increase in the size of the fat globules in both breeds, and that there was an increase in the number of the globules per cu.mm. in the Friesian cows.

To assess the extent and significance of these relationships the respective correlation

coefficients were calculated and tested for significance by using the '*t*' test as applied to correlations. The results were not significant for either variant because, although on the average the higher fat percentages were accompanied in both breeds by an increase in the fat globule diameter and in the Friesian cows by a rise in the number of globules per cu.mm., there were some cows in both breeds in which the higher fat percentages appeared to be accounted for by either an increase in fat globule diameter or an increase in the number per cu.mm., with correspondingly low figures for the other variant.

GENERAL CONCLUSIONS

Although the results were not significant, from an examination of the data recorded it would appear that, on the average, variations in the fat percentage of the milk of Friesian and Ayrshire cows were accompanied by corresponding changes in the diameter of the fat globules, and that in cows of the Friesian breed there were also, on the average, similar changes in the number of fat globules per cu.mm. It was, however, evident that there were some individuals in which variations were entirely or mainly accounted for by changes in either the fat globule diameter or the number of fat globules per cu.mm., and it is due to these individuals that the general trends were not significant.

SUMMARY

Milk samples were collected, between 17 April and 30 May 1956, from fifty-six Ayrshire and forty Friesian cows, which had been calved for between 45 and 165 days, when they were running out at grass day and night. The results showed that differences in the fat percentages of their milk were accompanied by corresponding variations in the average diameter of the globules in both breeds and in the numbers of globules per cu.mm. in the Friesian cows. These changes were not significant for either variant because there were some individuals in which changes in fat percentage appeared to be accounted for by either an increase in fat globule diameter or an increase in the number per cu.mm. with correspondingly low figures for the other variant.

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672. VARIATIONS IN THE SOLIDS-NOT-FAT CONTENT OF MILK. INVESTIGATIONS INTO THE NATURE OF THE SOLIDS-NOT-FAT PROBLEMS IN THE WEST MIDLANDS

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(With 2 Figures)

INTRODUCTION

The decline in the compositional quality of milk in the past fifty years has been the subject of much study. Bartlett & Kay (1) state that since 1920 the average composition of the nation's milk fell from 8·90 to 8·65 % solids-not-fat (S.N.F.), and emphasize the importance of this in terms of high-quality human food. A recent paper by Provan (2) gives the average composition of milk for England and Wales as 3·6 % butter fat (B.F.) and 8·7 % S.N.F., but milk from the west Midlands had an average composition of 3·4 % B.F., and only 8·57 % S.N.F. During certain months of the year, over 40 % of producers' bulk samples in this area fell below 8·5 %. That a serious solids-not-fat problem exists in the west Midlands is confirmed both by the dairies and by the numerous requests for advice received from individual milk producers. This paper gives the results of an investigation into the nature of this problem and an attempt to assess the main factors responsible.

NATURE OF THE INVESTIGATION

Eleven herds from the main milk-producing areas in the west Midlands were originally chosen to take part in the investigations which started in September 1954 and continued until August 1956. For various reasons four herds were subsequently found to be unsatisfactory and most of the information in this paper relates to the seven remaining herds. It is pointed out that these farms were chosen solely on the basis of a known history of low solids-not-fat to a greater or less degree, and were considered typical of the poor-quality milk-producing farms in the west Midlands province. Brief details of the herds included in the investigation are given in Table 1. Standards of management varied between herds, but would be described in general terms as average to good. Each farm attempted to make

Table 1. *General information on the herds under investigation*

Code letter of herd	Location	Breed	Av. size of herd	Average composition (S.N.F. %) 1953–54	Average composition (S.N.F. %) 1954–55	Bulk milk 1955–56 (S.N.F. %)
A	Cheshire	Friesian*	20	8·49	8·57	8·51
B	Cheshire	Ayrshire	20	8·58	8·73	8·63
C	Staffordshire	Friesian	25		8·20	8·21
D	Shropshire	Friesian*	50		8·60	8·49
E	Shropshire	Friesian	45	8·38	8·33	8·17
F	Warwick	Mixed	10		8·40	8·42
G	Warwick	Friesian	10	8·40	8·47	8·58

* Also contained a few Channel Islands cattle.

good use of home-grown foods—with one exception all made silage and most grew some kale. All made considerable use of grass. Winter-feeding programmes again varied, farms B and E obtaining maintenance and 1 gal. from bulk foods, whilst the remainder relied on roughage for maintenance only. Both home-mixed and proprietary concentrates were used for milk production. Farms were visited at least once a month by N.A.A.S. officers who kept records of feeding and management. Samples of milk were taken from individual cows each month, and samples of bulk milk were collected where possible. Evening milk only was sampled mainly for the convenience of the farmer, there being little difference between % S.N.F. of evening and morning milk. In a review article Bailey (3) quotes a mean difference between morning and afternoon milk of 0·07 % S.N.F.

Samples were despatched to the laboratory by train and usually arrived during the following day. As a precautionary measure a little formalin was added to the bottles before leaving the laboratory. On arrival each sample was examined for butterfat and solids-not-fat. In addition, every 3 months samples were further analysed for crude protein and lactose. (Because of staff shortage it was not possible to estimate lactose during the last months of the investigations.)

METHODS OF ANALYSIS

Butter fat was determined by the Gerber butyrometer. Total solids were determined gravimetrically. About 5 g. of milk were weighed into a flat-bottomed dish and dried according to a modified procedure of that recommended by the Society of Public Analysts (4). Solids-not-fat were calculated by difference. Crude protein was determined by the Kjeldahl technique ($N \times 6{\cdot}39$). Lactose was estimated by a Lane & Eynon titration after precipitation with zinc acetate and potassium ferrocyanide, and the results were expressed as the anhydride.

RESULTS

General

Average S.N.F. figures for bulk herd milks are given in Table 1. Results for 1953–54 and some for the period 1954–55 were obtained from the respective dairies. Lactation averages of S.N.F. were calculated for individual cows based on the mean of monthly tests only. Only two herds were officially recorded, and the rest operated a voluntary scheme in a more or less regular manner or did not record at all. In fact one of the secondary aims of the investigation has been to show the value of milk recording. Since reliable estimates of yield were not always available, weighted averages were not calculated. Fig. 1 is a histogram showing the frequency distribution of all lactation averages for S.N.F.

The effect of season

The period September 1954–August 1956 could hardly be described as typical with regard to weather conditions and was perhaps more one of extremes. The wet autumn and winter of 1954–55 were followed by a hot dry summer in 1955. Spring 1956 was exceptionally dry and late, followed by a very wet spell in July and August. No attempt has been made to separate the effects of season and stage of lactation as done by Waite, White & Robertson (5), who quote the effect of season only on milk quality to be of the order of $\pm 0{\cdot}1$ %, and conclude this is due mainly to changes in management. Seasonal

variations in milk quality agree broadly with what one would expect from previous observations. The poorest quality milk was produced during the late winter months, and the best quality during May and June. Table 2 shows the percentage of all milk samples received which fell below 8·5 and 8·0% S.N.F. respectively for each month of the year. Information on calving dates is given in Table 3. These are not evenly distributed throughout the year and in general show a predominance of autumn calving cows.

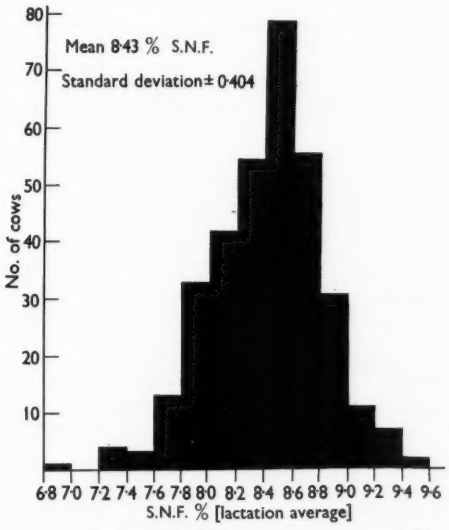

Fig. 1. Frequency distribution of lactation averages of S.N.F. (all cows 1954–56).

Table 2. *Seasonal distribution of low-quality milk*

Month	Jan.	Feb.	Mar.	Apl.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
Total no. samples received	313	316	340	360	327	366	324	287	281	293	307	297
Percentage below 8·5% S.N.F.	59·8	67·8	70·2	69·6	49·4	43·1	56·0	55·8	64·8	46·2	61·1	58·5
Percentage below 8·0% S.N.F.	24·0	23·4	25·6	28·8	15·3	13·4	22·2	22·3	17·4	17·7	21·4	24·2

Table 3. *Distribution of calving dates*

Month	Jan.	Feb.	Mar.	Apl.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
No. of cows calving	33	24	38	19	22	8	2	32	63	54	34	35
%	9·1	6·6	10·4	5·2	6·0	2·2	0·6	8·8	17·3	14·8	9·3	9·6

The effect of stage of lactation

This factor was examined on a herd basis. Stage of lactation curves for S.N.F., crude protein and lactose were prepared from information collected from the four largest herds (A, C, D and E). The animals making up these herds were almost entirely of the commercial Friesian type. Separate curves were prepared for the periods September 1954–August 1955, and September 1955–August 1956 (with the exception of the lactose curves which refer to 1954–55 only). These are shown graphically in Fig. 2. In general, the shape

of these graphs follow trends similar to those obtained by previous workers (5, 6, 14). The lactation curves for each herd tend to have their own particular shape which is repeated from year to year. Changes in S.N.F. content of milk with advancing lactation are to a large extent reflexions of the changes in crude protein. Lactose appears to be the more constant constituent, but tends to decrease slightly with advancing lactation.

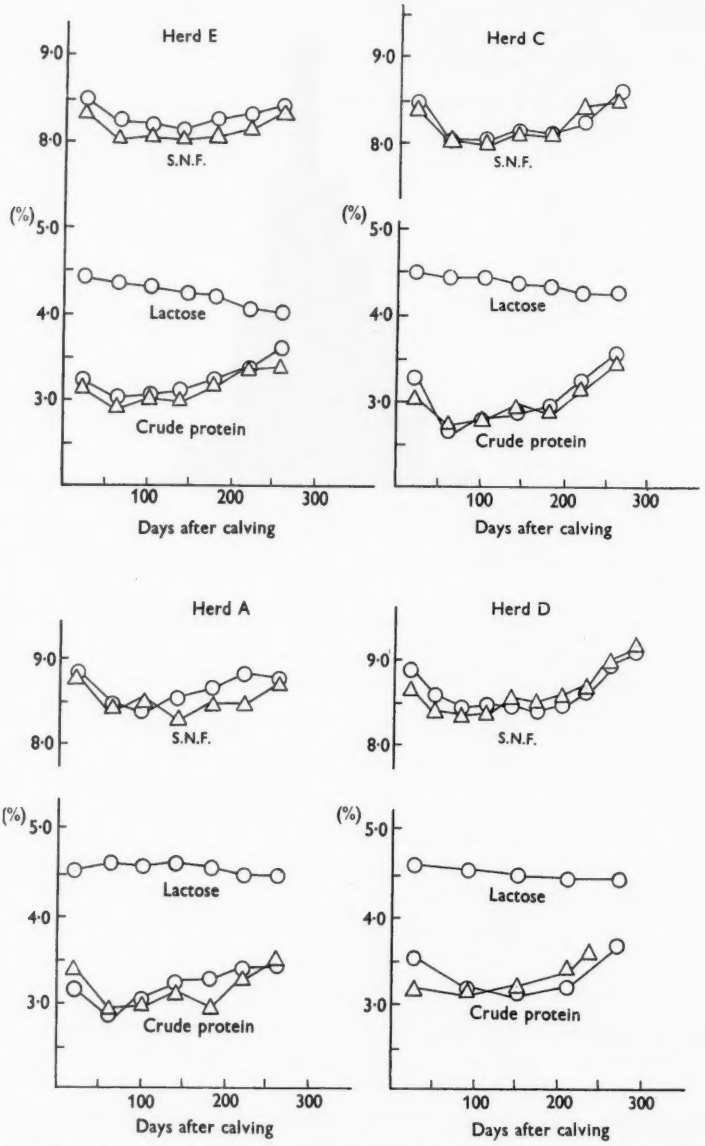

Fig. 2. Stage of lactation curves for S.N.F., lactose and crude protein (herds A, C, D and E). ○, period 1954–55; △, period 1955–56.

The effect of age

The lactation averages for S.N.F. of all cows were grouped according to lactation number and the mean was calculated for each group. These results are shown in Table 4.

Table 4. *Effect of age on milk composition*

Lactation no.	No. of cows	S.N.F. (%)	Age distribution (%)	Age distribution of cows in herds M.M. Bd. 1947 (15) (%)
1	89	8·63	26·4	25·6
2	88	8·54	26·2	21·6
3	55	8·40	16·4	16·9
4	46	8·21	13·7	13·8
5	27	8·22	8·0	9·2
6 and over	31	8·07	9·3	12·9

Actual age distribution is compared with figures for the national herd given by the Milk Marketing Board in 1947. Considering the small size of the sample, agreement is satisfactory.

The results confirm the regression of S.N.F. with age to be of the order of 0·1 % for each successive lactation (1).

The effect of feeding on milk quality

In recent years, following the work of Rowland and others (7, 8), considerable emphasis has been placed on the plane of nutrition, especially of energy intake and its relation to milk quality. In this investigation special attention was paid to the feeding of the dairy cows. The officer in charge of each herd on his monthly visit recorded details of feeding and verified this by occasional check weighings of food. Changes in feeding were advised if thought necessary, to bring the rations in line with Woodman's Standards (9). It is admitted we only have the farmers' assurance of what was fed for most of the time, but both from the condition of the cattle and from total milk production, we are confident that no gross underfeeding took place. Any herd that was not considered satisfactory in this respect was not included in the investigation during its second year. In spite of continued checks on feeding, the remaining herds continued to produce poor-quality milk (see Tables 1 and 2). It is generally agreed that the major effect of undernutrition on milk quality is to reduce the crude protein content of the milk (7, 8). Hence one would expect that any herd that was underfed would produce milk of a relatively lower crude protein content. For herd A (slightly below average for Friesians) and herds C and E (considerably below average for Friesians) the mean crude protein expressed as a percentage of the S.N.F. of the milk produced was 37·3, 37·1 and 37·7 respectively. A similar calculation from data given by Waite *et al.* (5) for Ayrshire milk gives a figure of 37·4 %.

It has recently been shown by Rook (10) and Holmes, Waite, MacLusky & Watson (11) that feeding extra starch equivalent (S.E.) in excess of Woodman's Standards resulted in an increase in the S.N.F. fraction of the milk produced. In order to verify these findings and as a further check on the adequacy of the feeding on the farm, a simple feeding trial was carried out at four centres during the late winter of 1955–56. Animals were selected as far as possible in pairs and the pairings were based on similar (*a*) age and breed, (*b*) stage of lactation (cows having calved 2–6 months only (6)), (*c*) yield, (*d*) S.N.F. (from previous records).

Treatment consisted of feeding one of the pair an additional 4 lb. maize per day (≡3 lb. S.E.) and was allocated by the toss of a coin. The trials continued for at least 6 weeks, and the final results were calculated from the average S.N.F. % for the last 14 days. Details are given in Table 5. A slight but not significant response was obtained from centre B only.

Table 5. *Results of feeding extra starch equivalent*

Farm	No. of pairs	Treatment	Mean S.N.F. % Start	Mean S.N.F. % Finish*	Mean, gain or loss (%)	Diff. of means	S.E. of diff. of means
B	3	Control expt.	8·28	8·18	−0·10	+0·18	0·11
			8·07	8·15	+0·08		
D	4	Control expt.	8·02	8·16	+0·14	−0·02	0·08
			8·17	8·29	+0·12		
E	6	Control expt.	7·54	7·67	+0·13	+0·06	0·10
			7·65	7·84	+0·19		
F	2	Control expt.	7·83	7·77	−0·06	−0·10	0·32
			8·10	7·94	−0·16		
Total	15	Control expt.	7·85	7·92	+0·07	+0·03	0·06
			7·93	8·03	+0·10		

* Average of results for the last 14 days.

Disease

It has been recognized for some time that diseases of the udder can affect milk composition, and it was with this in mind that inquiries were made about the incidence of mastitis in the herds under investigation. Farmers admitted an occasional outbreak of clinical mastitis, but did not consider the problem to be more serious than one would normally expect.

In order to check the incidence of subclinical infection, individual samples of fore-milk were taken by the Veterinary Investigation Officer for bacteriological examinations. These tests revealed that with the exception of one herd the incidence of *Streptococcus agalactiae* was not abnormal. This herd showed a high incidence of *Str. agalactiae* on two successive occasions, but unfortunately the farmer refused to co-operate in the eradication of the disease by veterinary treatment and the farm was subsequently dropped from the investigation.

DISCUSSION OF THE RESULTS

It can be seen from Table 1 that S.N.F. averages vary from herd to herd. Whilst some have bulk milk figures below 8·5 % S.N.F. practically all the year, for others it is merely a seasonal problem. The herds producing the poorest quality milk are of the Friesian type. In general milk quality was poorer in 1955–56 than in 1954–55. The exceptionally dry summer and autumn of 1955, and the late and very dry spring of 1956, might in some measure contribute to this. On the other hand, fodder supplies were much superior both in quantity and quality during the winter of 1955–56 than in 1954–55. The wide fluctuations of lactation average around a mean of about 8·5 % S.N.F. and the normal distribution of these averages is typical of any biological variable. However, the distribution is slightly skew, there being a disproportionately high number of cows giving a lactation average of 7·8–8·2 % S.N.F. The fact that some 70 % of individual cow samples fell below the minimum legal standards for S.N.F. during the late winter months illustrates the severity of the problem at its worst. Figures of a similar order have been reported by Nicholson &

Lesser in 1934 (12)—64 % of all samples examined were deficient in S.N.F. Throughout the year a considerable proportion of individual cow samples fails even to reach 8·0 % S.N.F.

The herds in the investigation followed as far as possible a policy of autumn calving, and the peak of some 51 % calving down during the months of September to December no doubt contributes in some measure to the high proportion of substandard milk produced from January to April in the following year. This is particularly important in herd D, where some 60 % of the total annual calvings occur during the 3 months August to October. Seasonal trends in general followed a normal pattern, the all-round improvement due to spring grass being the most noticeable feature. The magnitude of this increase was thought to be correlated with the quality of the spring grazing. Stage of lactation curves again followed the normal pattern, in as much as they tend to be the inverse of the yield curves. Hence the highest yield during the lactation tends to be associated with the poorest quality, and this can be an important factor in high yielding small herds. The shape of the lactation curve appears to differ between herds as it has been shown to differ in general shape between breeds (14). Bailey (6) has pointed out the effect of pregnancy on the shape of the lactation curve, and it can be said that the great majority of cows included in the investigation were not barren.

As stated previously, the age distribution of the dairy cattle in the investigation agrees quite closely with Milk Marketing Board figures for 1947 (15). In fact, it would appear that the sample contained rather more young cows than the national herd. This may in part be due to culling of older cows on the basis of S.N.F. records compiled during the investigation, but in any case the problem cannot be attributed to age alone. The regression of S.N.F. with age gives an average figure of 0·11 % per lactation and is in agreement with the generally accepted figure of 0·10 % (1). Whilst it is not claimed that the exact rations fed to the cattle were actually known, a close check was kept on feeding, and occasional changes were advised if necessary. In general, the cattle would be described as in good working condition, and no herd was noticeably underfed. Recent research has cast some doubt on the adequacy of Woodman's standards in terms of starch equivalent especially for high-yielding cows. Holmes *et al.* (11) have obtained increases in both yield and S.N.F. % by feeding starch equivalent at approximately 17 % in excess of Woodman's Standards to Ayrshire cows. However, in short-term trials we were unable to obtain any significant increase in S.N.F. by feeding an extra 3 lb. of starch equivalent during the late winter period. It is interesting that a small response was obtained from Ayrshire cattle. Although the crude protein percentage of the milk from individual cows often falls well below 3 %, it does not appear to be disproportionately low bearing in mind the low level of S.N.F. Gross underfeeding has been shown to lower the S.N.F. % of milk by only 0·2–0·3 %. Whilst feeding might account in some measure for seasonal variations in some herds, it was not a major factor in the herds under investigation. Very little information could be obtained from the co-operating farmers on the breeding records of their cows, but what little we have confirms the conclusions of Robertson *et al.* (13) that S.N.F. is strongly inherited. Using approximately 500 dam daughter comparisons, Robertson, Waite & White (13) estimated the heritability of S.N.F. to be of the order of 0·5 for the Ayrshire breed. The most striking evidence of the importance of heredity was obtained in this investigation from one of the smaller herds, in which the S.N.F. lactation averages of two daughters and four grand-daughters of a certain bull (nine lactations in all) were all low in relation to age of cow. Despite the fact that four out of the nine were heifer lactations,

the average figure was only 8·22% S.N.F. Such evidence of course is very meagre and perhaps not very convincing, but it does indicate the likelihood of the sire's influence upon the quality of his daughters' milk.

SUMMARY

1. During the period September 1954–August 1956 monthly samples of milk were obtained from individual cows in herds selected because of known history of low solids-not-fat. Records were kept of general feeding and management. Milk was analysed for total solids, butter fat and S.N.F. (by difference). Crude protein and lactose were also estimated at three-monthly intervals.

2. Mean lactation averages were calculated for S.N.F. These showed a normal distribution around a mean of approximately 8·5%.

3. Throughout the period a high proportion of samples failed to reach 8·5%. The seasonal effect is at its worst in March and April when 70% of the samples were below 8·5%.

4. Effect of advancing lactation (uncorrected for the effect of season) agreed broadly with previously published work but marked differences in the shape of the lactation curve were found between different herds. Variations in S.N.F. are largely a reflexion of variations in crude protein.

5. Age distribution of the cows agreed favourably with published figures for the national herd, and a mean regression of S.N.F. with age gave 0·11% per year.

6. Supervision of the feeding of the herds in the investigation gave no measurable improvement in S.N.F. and no significant response was obtained from short-term trials where extra S.E. was fed.

7. There was no undue incidence of disease of the udder, either clinical or subclinical.

8. Little information could be obtained on breeding, but it is concluded that the major cause of the problem is the genetically poor potentialities of the cows as judged from the average composition of the milk they produce.

If this conclusion be accepted, it is clearly of importance to test individual cow's milk for S.N.F. as well as for fat, especially in breeding herds. A start could be made with heifers, and only those producing high quality milk used for the selection of further breeding stock.

We wish to acknowledge the valuable assistance of the many people who made this investigation possible, and in particular to co-operating farmers who allowed us to sample their herds. In addition, we wish to thank those N.A.A.S. officers who were responsible for the collection and analysis of milk samples.

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673. BACTERIOLOGY OF CHEDDAR CHEESE

A STUDY OF STARTER ORGANISMS IN MANUFACTURE AND MATURING

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(With 1 Plate and 2 Figures)

INTRODUCTION

In the microflora of maturing Cheddar cheese there is a sequence of changes in which other species of bacteria, the lactobacilli in particular, replace the initial flora of streptococci.

While much work has been done on the role of lactobacilli in cheese maturing, the information regarding population trends of starter organisms and their influence on maturing is sparse and inconclusive.

Harding & Prucha (1) showed that *Streptococcus lactis* occurred in large numbers in green Cheddar cheese. Hastings, Evans & Hart (2), in studies of Cheddar cheese, found that the flora consisted of 90% *Str. lactis* after 100 days. Allen & Knowles (3) found that lactic streptococci predominated in cheese for the first 19–20 weeks. Whitehead & Sherwood (4), using *Str. cremoris* in a study of Cheddar cheese, found that the starter died out extremely rapidly within 14 days, being replaced by lactobacilli. The probable maximum starter population was 130 millions/g. Runov (5), working on Yaraslav, Soviet and Volga cheese, found that lactic streptococci reached a maximum population of 3000–3900 millions/g. Garey, Foster & Frazier (6) showed that the starter population in brick cheese reached a maximum of 1000–4000 millions/g. within 24 hr. of dipping. Janoschek (7), in a study of Emmenthal cheese, showed that streptococci increased during the manufacture of curd, and reached a maximum population in the press and thereafter steadily declined. Deane & Anderson (8) observed maximum counts in Cheddar cheese at one day, the numbers decreasing with age. *Str. lactis* made up 90–99% of the persisting flora. Knudsen & Sørensen (9), in studies of continental cheese, found that the starter population had died out within 36 days.

The aim of the present work was to examine population trends in Cheddar cheese made with individual strains of *Str. lactis*, *Str. cremoris* and *Str. diacetilactis*. Certain biochemical tests were carried out, partly for classification and partly to determine whether there was a relationship between population trend and physiological characteristics.

EXPERIMENTAL

General

The milk used was from a Friesian and Red Poll herd and had a methylene-blue reduction time of over 7 hr. The milk was pasteurized at $163 \pm 1°$ F. for 16 sec. Complications due to bacteriophage were eliminated by the use of water-seal lids (10), and by other appropriate precautions. (All cheese gave negative results when tested for bacteriophage.)

Method of manufacture

Two per cent of starter and 5 oz. of rennet per 100 gal. of milk were used. Whey was run at 0·15% acidity, the curd stirred twice while drying, and milled at between 0·6 and 0·7% acidity. Salt was added at the rate of 3½%. The 40 lb. cheeses were kept at 50° ± 3° F.

Physiological studies

The origin and phage relationships of the cultures used are given in Table 1.

Cultures were prepared in a slightly modified form of Hunter's broth (12), hereafter called H.P.B., and in skim-milk. Cultures were incubated at 30° C. for 18 hr., except for C13 and HP strains which were incubated at 25° C.

Table 1

Strain	Local name	Origin	Phage relationship
Streptococcus cremoris	HP	New Zealand	hp
	KH	New Zealand	kh
	ML1	England	ml1
	C3	England	c3
	C7	Australia	c7
	C13	Australia	c13
Str. lactis	C6	Australia	c10
	C4	Australia	c2, c3, c8
	C2	England	c4
	C8	England	c2
	C10	England	c6
Str. diacetilactis	DRC1	Australia	drc1
	DRC3	Australia	drc3
	DRC4	Australia	drc1

Physiological tests

The physiological tests applied to the cultures were designed, in addition to purposes of classification, to shed light on characteristics which are likely to affect their survival in cheese. Growth of all strains was tested in H.P.B. containing 2, 4 and 6·5% salt, and, because of its significance to cheese maturing, also in litmus milk at these salt concentrations. Cultures were grown at 10, 40 and 45° C. in H.P.B. and in milk.

The growth of all strains in H.P.B. containing 0·01 and 0·1% methylene blue was studied. Following the report by Davis (14) that sodium lactate serves as a differential medium for *Str. lactis* and *Str. cremoris*, all strains were tested for growth in milk containing 2 and 4% sodium lactate.

The above tests were made over a 5-day period. Ammonia production was tested after adequate growth in H.P.B. For the Voges–Proskauer reaction (13) a tryptone–yeast extract–lactose–citrate medium was used, and a check test was carried out in milk.

The morphological characteristics were examined in 18 hr. milk culture.

All strains were incubated at 30° C., excepting C13 and HP, for which 25° C. was known to be a more suitable temperature.

Study of starter bacteria during manufacture

In studies of maturing Cheddar cheese, it was found that maximum populations of starter bacteria occurred ex-press (18 hr.). The bacterial population at this stage was found to vary widely according to the particular starter strain. Variations in population at

earlier stages of cheese manufacture were therefore investigated for the cultures listed in Table 2.

Samples were taken of milk or curd: (*a*) immediately following the addition of starter, hereafter called 'setting'; (*b*) at the end of running the whey; (*c*) approximately halfway through Cheddaring (0·4% acidity); (*d*) following the milling operation; (*e*) from the cheese after 20 hr. in the press.

Counts were made immediately after sampling.

Table 2. *Starter strains used during manufacture and maturing*

Manufacture		Maturing	
Strain	No. of replications	Strain	No. of replications
C2	—	C2	—
C6	—	C4	1
C10	—	C6	1
DRC1	3	C8	—
DRC3	1	C10	—
C3	1	DRC1	—
C7	—	DRC3	3
C13	5	DRC4	—
HP	—	C3	1
ML1	2	C7	1
		C13	1
		HP	—
		ML1	—
		KH	—

Methods of population estimation

The appropriate dilutions were in all cases plated out in duplicate using tryptone-glucose–extract agar (Difco) with 1% skim-milk added. The bacterial populations were counted after 4 days at 30° ± 0·5° C.

As a check on the estimation of bacterial population by plate count, a number of samples were examined by the breed method for total count. For cheese curd the 1:100 dilution was found most suitable.

Dilution technique for bacterial titre. To check whether all viable bacteria were estimated by plate-count technique, 1 ml. portions of suitable dilutions of the samples in Ringer's were added to 9 ml. of litmus milk and incubated at 30° C. up to 5 days (for C13 and HP 25° C. was used). Five tubes at each of three dilutions were taken.

Study of bacteria distribution in the curd. Samples were taken at the beginning of Cheddaring and at milling, and a few ex-press. Microtome sections approximately 20 μ in thickness and 0·1 cm.^{2} in area were cut after freezing the sample with liquid CO_2. The sections were stained by Newman's method and examined microscopically under high dry and oil immersion, to study distribution of the micro-organisms. Photo-micrographs ($\times 1000$) were taken of sections of each cheese at the milling stage.

Starter study during maturing

Core samples were taken aseptically from the cheese beginning at one day, and thereafter as required. In general, cheeses were examined weekly for the first 5 weeks, thereafter approximately fortnightly until 3 months old, thence at approximately monthly intervals. The sampling ceased when the starter bacteria had died out or represented a

minor and static proportion of the population. The bacterial populations were determined by the plate count method immediately after sampling by taking a 5 g. representative sample of the core and treating in the same way as the curd samples. Table 2 lists the cultures studied during maturing. In many cases observations were confirmed by counts on duplicate cheeses.

To determine the proportion of starter bacteria in the total population, twenty-five colonies were chosen at random from suitable plates, subcultured in litmus milk and incubated at 30° C. The litmus milk cultures showing acid decolorized clot after 18 hr. were assumed to be starter organisms. All others were smeared after 48 hr. incubation, stained by Newman's method and examined to determine the presence of slow acid-producing starter organisms and other microflora.

RESULTS

Physiological studies

The physiological and morphological characteristics of the 14 cultures are given in Table 3. The results obtained with temperature tolerance in broth is in accord with accepted information. It is interesting to note that *Str. diacetilactis* appears to grow better in H.P.B. than in skim milk. Table 3 shows that the strains were far more salt-tolerant in milk than in broth, 6·5% salt inhibiting the growth of the *Str. lactis* and *Str. diacetilactis* strains in broth, but not in milk. C13 and HP cultures show less tolerance to salt and methylene blue than the other *cremoris* strains.

Davis (14) considered lactate concentration as a factor in the inhibition of streptococci in cheese, because he obtained inhibition of *cremoris* and *lactis* by respectively 2 and 4% sodium lactate in broth. It was considered that in this work milk was a more appropriate medium. Contrary to Davis, it was found that the streptococcal strains used in this study were tolerant to 2 and 4% sodium lactate in milk, indicating that this factor is unlikely to be important in inhibiting streptococci in cheese.

The study of morphology of the fourteen strains in milk indicated that the main difference was that of chain length. All *cremoris* strains showed long chains. The range of cell size is similar for all groups.

Studies in manufacture and maturing

In spite of all efforts to keep manufacturing conditions constant some variations did occur, but it was considered that these would not invalidate the comparison of starters. Table 4 outlines the ranges and averages obtained.

Manufacture studies

Trend in population

Counts of starter organisms at the several stages of manufacture are given in Table 5. The *cremoris* strains were found to have lower populations throughout than did *lactis* and *diacetilactis* strains. There is also a population difference, lesser but distinct, between the latter. There were differences in count between individual strains of each species. Examination of smears of ground material at the 1:100 dilution stage of preparation showed that the long and short chains of *Str. cremoris*, and the short chains of *Str. lactis* and

Table 3. *Differential characteristics of* Streptococcus lactis, Str. cremoris, Str. diacetilactis

Starter		*Str. lactis*					*Str. cremoris*						*Str. diacetilactis*			
		C2	C4	C6	C8	C10	C3	C7	C13	ML1	HP	KH	DRC1	DRC3	DRC4
Growth in broth at	10° C.	+ +	+ +	+ +	+ +	+ +	+	+	+	+	+	+	+	+	+
	40° C.	+ +	+	+ + +	+ +	+ + +	−	−	−	−	−	−	+ +	+ + +	+ +
	45° C.	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Growth in milk at	10° C.	+ +	+ +	+	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +
	40° C.	+ +	+	+ + +	+ + +	+ + +	−	−	−	−	−	−	−	−	+
	45° C.	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Salt tolerance	Broth 2%	+ + +	+ + +	+ + +	+ + +	+ + +	−	+ +	−	+ +	−	+ +	+ + +	+ + +	+ + +
	Broth 4%	+ +	+ + +	+ + +	+ + +	+ + +	−	−	−	−	−	−	+ + +	+ + +	+ + +
	Broth 6·5%	−	−	−	+ +	−	−	−	−	−	−	−	−	−	−
	Milk 2%	+ + +	+ + +	+ + +	+ +	+ +	+ +	+ +	+	+ +	+	+ +	+ + +	+ +	+ +
	Milk 4%	+ +	+ +	+ +	+ +	+ +	+	+ +	−	+	−	+	+ +	+	+
	Milk 6·5%	+	+	+	+	+	−	−	−	−	−	−	+	+	+
Voges-Proskauer	Broth	−	−	−	−	−	−	−	−	−	−	−	+	+	+
	Milk	−	−	−	−	−	−	−	−	−	−	−	+	+	+
Ammonia production		+	+	+	+	+	−	−	−	−	−	−	+	+	−
Methylene blue	0·01%	+ + +	+ + +	+ + +	+ + +	+ + +	+	+ +	−	+ +	−	+ +	+ + +	+ + +	+ + +
	0·1%	+ +	+ +	+ +	+ +	+ +	−	−	−	−	−	−	+ +	+ +	+ +
Sodium lactate in milk	2%	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ +	+ + +	+ + +	+ +	+ +	+ + +	+ +	+ +
	4%	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+	−	+ +	+ +	+ +	+ +
Morphology 18 hr. in milk at 25° C.		Diam. 0·3–0·75μ. Round to oval. Single and pairs, few short chains					Diam. 0·3–1μ. Round to oval. Pair, short and long chains						Diam. 0·3–1μ. Kidney, round and oval. Pairs, short chains, few long chains		

Growth: + + + = good; + + = fair; + = slight; − = no growth.
N.B. Relative growth scores given are only applicable to comparisons within each test.

Table 4. *General manufacture details*

	Cutting acidity	Running acidity*	Drying acidity	Milling acidity	Salt in water (%)†	Water (%)
Av.	0·10	0·15	0·20	0·64	5·89	34·4
Range	0·10–0·13	0·15	0·17–0·26	0·60–0·70	5·02–6·50	32·7–35·2

* Acidity as percentage lactic acid.
† Estimated on maturing studies only.

Str. diacetilactis, had in general fragmented into single and paired cells. This means that all plate counts quoted in the table for curd samples are comparable.

The counts at the various sampling stages were averaged for each species. These averages expressed as logarithms are shown in Fig. 1. *Str. lactis* reaches its maximum (4500 millions/ g.) at about the half-Cheddar stage and maintains this population up to the last sampling stage at ex-press. *Str. diacetilactis* also reaches its maximum (2900 millions/g.) at half-Cheddar, but thereafter steadily declines. *Str. cremoris*, on the other hand, reaches its maximum (960 millions/g.) at milling and then declines in the same way as *Str. diacetilactis*. The growth rate of *Str. cremoris* up to half-Cheddar is more rapid than the other two species, which are comparable, but is slower between half-Cheddar and milling.

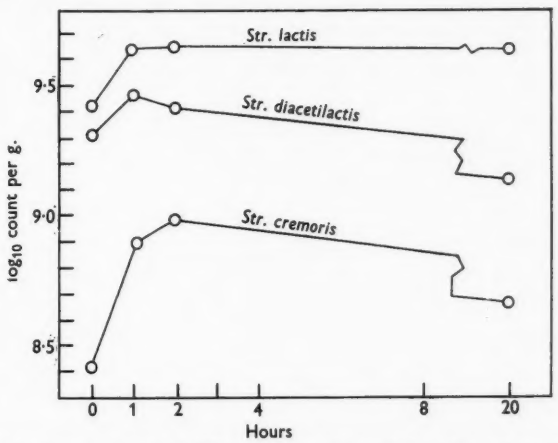


Fig. 1. Average counts at different stages of manufacture for each species.

Some populations were determined at setting. Table 5 shows the *cremoris* strains to have lower counts in general than *lactis* and *diacetilactis* strains. However, microscopic examination of the Breed sample at setting shows that in general the *cremoris* strains have longer chains. Since in diluting a milk sample for plating the individual cells are not separated to the same extent as in the grinding of the curd sample, the populations in curd cannot be compared directly with those in milk at the setting stage. For this reason the setting figures were not included in Fig. 1.

Variation of starter population within strains

It was observed that some strains showed lack of agreement in bacterial numbers for samples from the press. To determine the amount of variation, these strains (C13, DRC1, DRC3, ML1, C3) were examined on several occasions. Details are given in Table 5.

Reasonable agreement was obtained for most replicates, but some differences were observed. These, however, are considerably smaller than the differences in populations obtained for each species.

Table 5. *Starter population during manufacture*

(Counts in millions/g.)

Starter	Time* hr.	Time* min.	Setting	Running	Cheddar	Milling	Ex-press	Acidity
HP	5	40	19	68	640	225	41	0·64
MLI (i)	6	10	—	—	440	455	230	0·68
ML1 (ii)	6	10	—	245	435	520	300	0·63
ML1 (iii)	5	50	8·4	755	450	1325	210	0·63
C3 (i)	5		—	300	530	410	450	0·63
C3 (ii)	5	25	—	415	1000	890	—	0·69
C7	5	20	9·0	198†	1770	2900	910	0·66
C13 (i)	5	35	—	260	360	420	—	0·63
C13 (ii)	5	45	—	158	360	460	205	0·61
C13 (iii)	4	35	—	200	280	295	55	0·66
C13 (iv)	5	25	1·2	—	390	450	3·2	0·67
C13 (v)	4	40	2·3	290	360	1000	142	0·65
C13 (vi)	5		2	31†	139	310	10	0·66
DRC1 (i)	5	30	19	3000	3000	2730	1140	0·66
DRC1 (ii)	5	5	—	4000	5000	—	2170	0·70
DRC1 (iii)	5	5	—	2630	4250	3700	1460	0·60
DRC1 (iv)	5	30	36	2380	2750	3400	1220	0·68
DRC3 (i)	5		23	930	1170	2720	410	0·62
DRC3 (ii)	5	30	40	1080	2520	2150	1610	0·61
C2	5		—	2800	4250	4100	1400	0·70
C6	5	50	—	2650	3300	5050	6600	0·60
C10	5	15	45	2400	5500	4400	5000	0·66

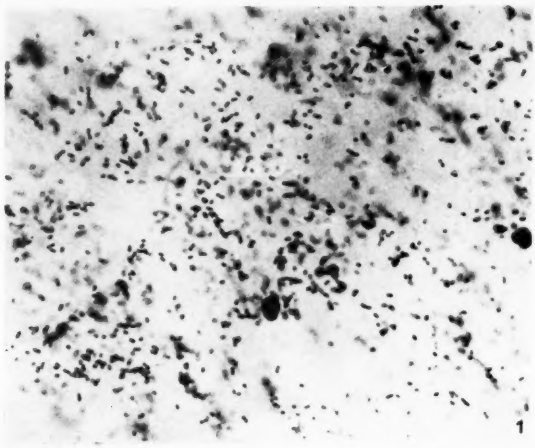
* Period from time starter added until curd milled.
† Considered erroneous results.

Acidity in relation to count

Table 5 records the times required to reach the milling acidity. These range from 4 hr. 35 min. to 6 hr. 10 min. The longer manufacturing times can in the main be attributed to periodic inhibition of individual starters by inhibitory substances in the milk. The populations reached at this milling acidity differed considerably. With *Str. cremoris*, the average population was 960 millions/g.; *Str. diacetilactis*, 2600 millions/g.; and *Str. lactis*, 4500 millions/g. (If the strain C7 were omitted, the average of the *cremoris* group would be much lower.)

The Breed count and dilution technique for bacterial titre

It was found that certain *cremoris* strains gave plate counts for some stages of manufacture which appeared out of line with the general trend and magnitude of populations obtained. The Breed count and dilution techniques were applied to see if these apparent anomalies were due to irregularities in plate method and/or to intrinsic variation in the organism. In evaluating these results the error of the Breed count must be remembered. It was also found that debris present in the ground curd made counting of cells difficult. The dilution technique used was that according to Finney (15). The dilutions and number of replicates used was limited by practical considerations. This meant that the estimate was subject to a large standard error. The figures quoted are estimates of the mean value. In Table 6 some of the counts by the three methods are recorded. C13 (vii) shows an absurdly



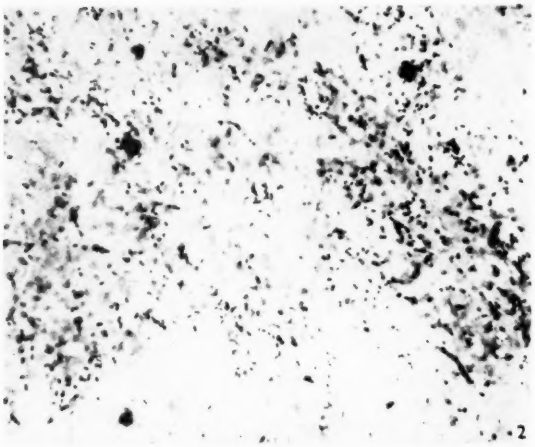


Fig. 1. *Streptococcus lactis*, strain C2. Fig. 2. *Str. diacetilactis*, strain DRC3. Fig. 3. *Str. cremoris*, strain H.P.

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low count by plate method. The dilution technique estimates given show that the count is much higher and more in line with other counts observed with this starter. The low plate counts obtained on this occasion are probably due to variation of this culture in its media requirements. C13 (vi) shows a similar but smaller variation, C13 (i), (ii), (iii), (iv), (v), as shown in Table 5, give plate counts which are more in keeping with expected values.

In comparison with the plate counts obtained for C7 at half-Cheddar, milling and express, the count of 198 millions at running is too low. Both Breed (625 millions) and dilution technique (1050 millions) counts indicate a higher figure. The other results given in Table 6 show reasonable agreement between the three techniques, indicating that in general the plate count was giving a reliable estimate of the population.

Table 6. *Comparison of plate count, Breed count and dilution titre*

(Counts in millions/g.)

Starter		Setting	Running	Cheddar	Milling	Ex-press
C13 (v)	P	2·3	290	360	1000	142
	B	2	260	290	960	165
	D	5·4	1150	310	1930	139
C13 (vi)	P	2	31	139	310	10
	B	1·7	100	150	240	40
	D	3·4	160	730	637	81
C13 (vii)	P	—	5	8	26	3
	B	—	—	—	—	—
	D	—	637	637	1050	—
DRC1	P	19	3000	3000	2730	1140
	B	15	2080	3800	4600	2800
	D	17·5	—	3000	3400	—
DRC3	P	23	930	1170	2720	410
	B	30	1000	2080	1760	1600
	D	34	1650	—	—	—
ML1	P	8·4	755	450	1325	210
	B	5·5	800	560	850	100
	D	5·4	810	—	1310	220
C7	P	9	198	1770	2900	910
	B	11	625	1730	2960	740
	D	—	1050	3350	—	1050
C10	P	45	2400	5500	4400	5000
	B	39	2000	6000	4250	5000
	D	—	2900	8000	8000	3000

P = plate count; B = Breed count; D = dilution technique count.

Study of cell distribution in the curd

Pl. 1, figs. 1–3, show typical distribution of starter organisms in the curd for the three species studied. Whereas *Str. lactis* and *Str. diacetilactis* show an even scattering of diplococci, *Str. cremoris* shows an uneven distribution with aggregation of chains into loose colonies. Since the milling sample showed this to advantage, it was selected for photographing. The size of the *Str. cremoris* colonies varied for the different strains, and also within the strain. The colony size ranged from 20 to 70μ in length and from 5 to 20μ in diameter. In some cases the *lactis* and *diacetilactis* species showed a few small aggregates of up to 5μ in diameter.

Maturing studies

An experiment was carried out to determine whether a single core sample would give a representative count of the whole cheese and also of the whole manufacture. Results are given in Table 7. Counts between cheese and within cheese showed sufficiently good agreement on three separate occasions to justify single-core sampling.

Table 7. *Reproducibility of plate count for cheese*

(Counts in millions/g.)

Sampled at days	Cheese 1		Cheese 2	
	Core 1*	Core 2*	Core 1*	Core 2*
15	1350	1940	1370	1820
32	500	680	440	490
63	—	28	17	22

* Average of duplicate plates.

Trend in population

Counts of total population and the proportion of starter organisms from 1 to 161 days are given in Table 8. It has been already shown that maximum populations are obtained during manufacture. In Table 8 it is shown that populations (ranging from 27 to 9000 millions/g.) are recorded at the first sampling (ex-press), and thereafter decline to reach, finally, a low and static figure.

Table 8 also reveals considerable variability within each species, e.g. the *lactis* strain C6 shows a maximum of 9000 millions/g., whereas C4 only attains 1400 millions/g. Similar variability is shown for *diacetilactis* strains (410–2800 millions/g.) and for *cremoris* (27–1800 millions/g.). The results of counts on replicate cheeses made using the same starter are given in Table 9.

At ex-press, strains of *cremoris* show a greater variability in starter population than do those of *lactis* (see also Table 8). With the former, sampling is taking place during a period of rapidly declining population and the count will therefore be largely dependent upon the precise stage of sampling. Table 9 also shows that the within-strain variability for *cremoris* is greater than that for *lactis*. The validity of the counts during maturing is confirmed by the close correspondence of duplicates given in Table 9. Previous experience in complete maturing studies of some of the above starters gave confirmation to the present studies. It was therefore considered that each species shows a reasonably specific population trend. In the case where the strain did not follow the expected population trend duplication was carried out over the original maturing period. It can be seen from Table 8 that starter C4 does not show the expected trend of *lactis* type. (The starter died out earlier than usual.)

Similarly, for C7 and to a lesser extent for C3, the ex-press populations are higher and persist longer than the other *cremoris* strains. Table 9 gives the duplication of these three starters and confirms the original population trend of the strain.

Fig. 2 gives logarithms of the average counts for each species at the different stages of maturing. *Str. lactis* shows much higher populations at comparable stages than *Str. diacetilactis* and *Str. cremoris*. The proportional decrease of population for each species is similar. With all three species the starter organisms form almost the whole population up to 21 days. With further ageing, in cheese made with *Str. cremoris*, the starter becomes a

rapidly decreasing proportion of the population, whereas with cheese made with *Str. lactis* and *Str. diacetilactis* the starter continues to represent a high proportion of the total population until a much later stage of maturing. It can also be seen that the fall is more gradual for *Str. lactis* and *Str. diacetilactis*.

Table 8. *Total population and percentage starter at different stages during maturing of Cheddar cheese*

(Millions/g.)

	Str. lactis					*Str. diacetilactis*			*Str. cremoris*					
Days	C6	C10	C8	C2	C4	DRC4	DRC1	DRC3*	C7	C3	ML1	KH	C13	HP
1	9000	4300	2500	1450	1400	670	2800	410	1800	690	500	100	40	27
	100†	*100*	*100*	*100*	*100*	*100*	*100*	*100*	*100*	*100*	*100*	*100*	*95*	*100*
8	5000	3500	2300	690	1500	480	370	430	1400	217	65	202	15	38
	100	*100*	*100*	*100*	*100*	*100*	*100*	*100*	*100*	*95*	*100*	*100*	*0*	*100*
15	4500	2900	2000	300	320	76	750	—	630	175	60	150	28	10
	100	*100*	*85*	*100*	*100*	*100*	*100*		*100*	*90*	*100*	*95*	*0*	*80*
22	7800	3200	1300	—	280	120	300	—	320	200	33	2	63	14
	100	*100*	*95*		*100*	*100*	*100*		*90*	*100*	*100*	*100*	*0*	*95*
29	4900	3200	2400	300	140	—	100	—	175	4·8	22	30	—	1·2
	100	*100*	*100*	*100*	*100*		*100*		*40*	*85*	*95*	*15*		*5*
36	4000	1830	—	139	—	100	—	—	—	11	6	30	191	1·5
	100	*100*		*100*		*100*				*40*	*80*	*20*	*0*	*0*
43	—	2020	650	450	25	—	105	—	83	8	14	30	—	—
		100	*90*	*100*	*85*		*95*		*5*	*50*	*100*	*15*		
50	1000	—	—	250	—	—	—	—	—	9	4·5	19	—	—
	100			*95*						*10*	*60*	*20*		
56	—	850	360	154	24	8·9	44	—	8	9	—	24	—	—
		100	*85*	*95*	*80*	*100*	*65*		*10*	*0*		*5*		
65	72	45	—	230	—	—	—	—	—	18	14	37	—	—
	70	*90*		*100*						*0*	*75*	*5*		
70	—	—	—	—	—	30	30	—	—	—	—	—	—	—
						90	*30*							
77	74	30	137	85	30	—	—	—	—	—	0·5	—	—	—
	35	*60*	*100*	*75*	*30*						*30*			
84	—	—	—	—	—	21	—	—	—	—	—	—	—	—
						65								
99	42	12	71	21	21	35	—	—	—	—	—	—	—	—
	0	*75*	*85*	*50*	*0*	*90*								
105	—	—	—	—	—	—	23	—	—	—	—	—	—	—
							0							
128	27	8·9	39	19	—	—	—	—	—	—	—	—	—	—
	0	*0*	*50*	*50*										
157	—	—	—	21	—	—	—	—	—	—	—	—	—	—
				15										
161	—	—	15	—	—	—	—	—	—	—	—	—	—	—
			0											
Manufacture times	5 hr. 55 min.	4 hr. 40 min.	4 hr. 40 min.	5 hr. 20 min.	4 hr. 20 min.	6 hr.	5 hr. 25 min.	5 hr.	5 hr. 20 min.	4 hr. 35 min.	5 hr. 10 min.	4 hr. 35 min.	4 hr. 45 min.	4 hr. 40 min.
Milling acidity	0·61	0·62	0·64	0·64	0·68	0·63	0·65	0·65	0·65	0·63	0·60	0·67	0·64	0·62

* Data incomplete for DRC3.

† Figures in italics represent percentage of total as starter organisms.

Cheese isolates

The litmus milk cultures obtained by picking twenty-five colonies at random from each cheese were examined (approximately 4000 cultures were studied). It was found that the isolates of each starter organism could be placed into two groups, viz. those clotting the milk after 18 hr. incubation at 30° C., (hereafter called fast substrain) and those which

Table 9. *The variation of count as given by different cheeses*

		Starter. Count in millions/g. at 1 day*						
C2	C6	C10	DRC1	DRC3	C13	HP	ML1	
1400	9000	5000	2800	1610	40	27	500	
1450	6600	4300	1460	410	105	41	230	
—	7000	—	2170	55	55	—	300	
—	—	—	1220	—	3·2	—	210	
—	—	—	—	—	142	—	—	
—	—	—	—	—	90	—	—	
—	—	—	—	—	10	—	—	

Age in days...		1	7	15	21	28	42	56
Starter count in millions/g.	C4	1400	1500	300	—	140	25	—
		1140	600	300	—	112	25	—
	C3	690	217	175	14	1	—	—
		450	—	—	—	—	—	—
		425	214	47	6	2	—	—
	C7	1800	—	630	175	—	—	8
		910	—	—	—	—	—	—
		890	—	470	300	—	—	14

* Some counts taken from Table 8 for comparison.

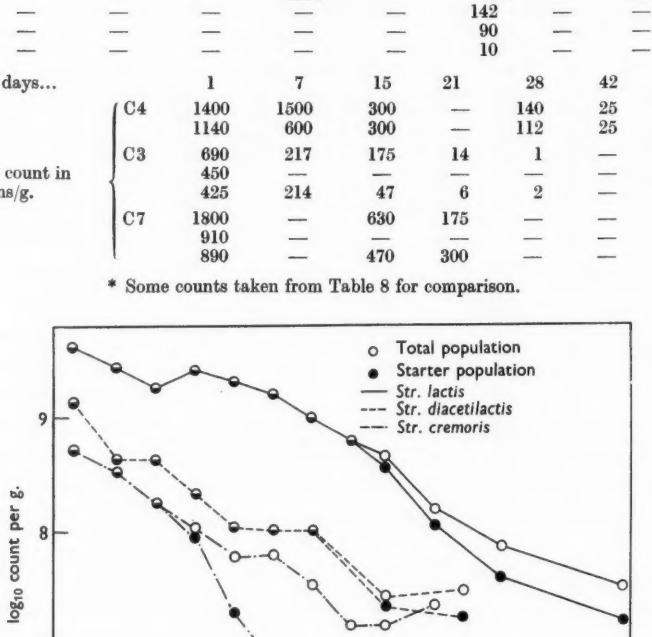

Fig. 2. A comparison of the mean total and starter counts for the three species during maturing.

produced lactic acid much more slowly (clotting time varying from 48 hr. to 14 days). These are hereafter called slow substrains. The total numbers of fast and of slow substrains isolated for each species at each sampling time were summed and are given in Table 10.

It can be seen that the proportion of fast substrains is considerably higher for *lactis* than for *cremoris*. In the case of *lactis* there seems to be some tendency for the proportion of slow substrain to increase as the cheese matures. With *diacetilactis* the figures given are averaged from only three strains. The fast-slow ratio for DRC1 and DRC3 is similar to that for *lactis*. But the average figures given, which show a considerable proportion of slow substrain, is due to the DRC4 strain. This strain shows a high percentage of slow substrain in the starter culture. This also probably accounts for its slowness in the vat.

Table 10. *Frequency of fast and slow strains isolated from the cheeses during maturing studies*

Days...	1	8	15	22	29	36	43	50	56	65	77	99	
Str. lactis	121	121	118	87	105	57	90	—	78	53	63	46	Fast
	2	3	4	15	19	22	1	—	10	10	11	7	Slow
Str. diacetilactis	58	41	41	38	—	—	—	—	—	—	—	—	Fast
	17	36	29	32	—	—	—	—	—	—	—	—	Slow
Str. cremoris	59	51	41	53	29	13	4	—	—	—	—	—	Fast
	43	62	75	60	26	24	35	—	—	—	—	—	Slow

The non-starter population was predominantely lactobacilli and micrococci. The proportion of micrococci and lactobacilli found in individual cheeses at different ages varied considerably. There seemed to be no regular sequence in the occurrence of the two types of organisms. The relative percentages of each type differed for cheeses made with *lactis* and *cremoris* starters. With *lactis* strains the micrococci constituted 20% of the non-starter population and lactobacilli 80%. For *cremoris* the proportions found were 40 and 60% respectively. The number of non-starter isolates for *diacetilactis* strains was too small to justify any inference from the data.

DISCUSSION

The manufacturing studies have shown that considerable differences occurred between the average maximum populations reached for the three common species of streptococci used in Cheddar cheese manufacture. The average rate of decline (persistence) of the three species in the maturing cheese also differed considerably. This variation could largely account for the inconsistencies in maximum counts and rate of decline of starter populations in cheese observed by previous workers (2)–(9). Inconsistencies in their results could also have arisen from the presence of bacteriophage: we have shown in another study (unpublished data) that the presence of low concentrations of phage, although not noticeably retarding acid production in manufacture, depressed the population at ex-press. In some of these cases the starter population recovered to give maximum numbers several days after manufacture.

Further within each species, the different strains show, within certain limits (especially in the case of *cremoris*), differences in maximum populations and in persistence in the maturing cheese. These limits appear to be characteristic for each species studied. Since differences in population between and within strains did occur, it was found that replication was necessary to ensure validity of results.

From the limited observations with Breed counts taken at 'setting', the initial population of *cremoris* strains appeared to be at least 50% of the cell count of *lactis* strains. On this basis the differences in maximum population (see Table 5) between the species must be due to a difference in rate of multiplication. It also appears that the most rapid increase of population occurs between 'setting' and 'running'. Since the rate of acid development is similar for widely different maximum populations, one must conclude that there are differences in the amount of acid produced per cell division. Thus *Str. cremoris* strains produce considerably more lactic acid per cell division than the other two species. There appears to be a smaller difference between strains of *Str. lactis* and *Str. diacetilactis*. To some extent this is supported by the evidence of Foter & Rahn (16), who found considerable

difference in the lactose requirement per cell division between *Str. lactis* and *Str. faecalis*. Further, Dorn & Rahn (17), using *Str. lactis* and *Str. thermophilus*, have shown that the fermentation rate is not necessarily related to optimum temperatures for multiplication and differed for each species.

In Table 11 the thirteen strains (DRC3 was omitted because of insufficient data) are placed in order of 'starter intensity' which was calculated arbitrarily from the maximum populations reached and the persistence of the organism in the cheese (see Tables 5 and 8). There are also listed for each strain the morphological and physiological characteristics: tolerance to salt, temperature, and methylene blue (see Table 3) and the average number of cells in the chain.

Table 11. *Relation of physiological characteristics of cultures to their persistence in cheese during maturing*

Order of starter intensity	Relative degree of: Salt tolerance	Temperature tolerance	Methylene blue tolerance	Average no. of cells per chain
C6	A	A	A	2
C10	A	A	A	2
C8	A	A	A	2
C2	A	B	A	2
C4	A	C	A	2
DRC1	A	D	A	5
DRC4	B	C	A	8
C7	C	E	C	9
C3	D	E	C	12
ML1	D	E	C	20
KH	D	E	C	15
HP	F	E	E	45
C13	F	E	E	90

A = most tolerant. F = least tolerant.

The tolerance scores given were placed into broad classes ranging from A to F. There appears to be high correlation between the four chosen characteristics and starter intensity. It is considered that chain length can be used as a suitable indicator of the starter's ability to persist in the cheese. These results also suggest that milk is a more suitable medium than broth for measuring salt tolerance of starter streptococci in relation to cheese.

Davis (14) and Sherwood (18) found that lactic streptococci and lactobacilli constituted 99% of the flora during the maturing of Cheddar cheese. Contrary to this, it was found in the present investigation that micrococci formed a considerable proportion (29%) of the flora. At some stages in maturing, they were the dominant types present.

The study of microtome sections gave a clear picture of the cell distribution in the curd. The fourteen cultures studied fall into two definite groups. Whereas *Str. lactis* and *Str. diacetilactis* show even scattering, *Str. cremoris* was unevenly distributed and occurred mainly as large colonies. It seems probable that the tendency for long-chain formation in *Str. cremoris* is responsible for the formation of colonies.

The results have shown that there is a big difference in bacterial population when *Str. lactis* and *Str. cremoris* are used as starters in cheesemaking. The higher populations and persistence of starter bacteria in the former compared with the much lower popula-

tions and rapid dying out of the organisms in the latter must produce a different environment for the growth of other micro-organisms. If cheese flavour is influenced by the growth of these secondary micro-organisms, the use of a *lactis* or *cremoris* starter must be a significant ecological factor in flavour development.

SUMMARY

1. The trends of starter bacteria population during the manufacture and maturing of Cheddar cheese have been investigated. Fourteen strains consisting of *Str. lactis*, *Str. diacetilactis* and *Str. cremoris* have been studied.

2. Physiological and morphological characteristics of the fourteen strains were examined.

3. Population studies during manufacture showed that the three species of starter organism attain quite different maximum populations and at different stages of the cheese-making process. *Str. lactis* (4500 millions/g.) and *Str. diacetilactis* (2900 millions/g.) reached their maximum half way through Cheddaring, whereas *Str. cremoris* (960 millions/g.) reached its maximum at milling.

4. *Str. lactis* was shown to maintain its maximum population until the cheese left the press, whereas *Str. diacetilactis* and *Str. cremoris* steadily declined after reaching their maximum.

5. Counts of the different starter cultures used showed considerable variation between and within strains, particularly in the case of *Str. cremoris*.

6. No relationship was shown to exist between acidity development and starter populations developed.

7. The study of cell distribution in the cheese curd has shown that whereas *Str. lactis* and *Str. diacetilactis* gave an even scattering of diplococci, *Str. cremoris* showed colony formation.

8. *Str. lactis* strains maintained a high but declining population during maturing of the cheese, the starter beginning to die out gradually after 8 weeks. *Str. diacetilactis* showed similar trends but at lower populations levels. *Str. cremoris*, on the other hand, gave much lower populations, the starter dying out rapidly after 2 weeks, and virtually disappearing at 8 weeks.

9. Persistence of starter bacteria in the cheese appears to be correlated with salt tolerance and chain length.

10. Starter bacteria isolated from the cheese made with *Str. lactis* have shown a low proportion of slow acid-producing types in contrast to a high proportion found with *Str. cremoris*.

11. The non-starter bacteria in the cheese were found to consist on the average of 30% micrococci and 70% lactobacilli. Negligible numbers of other genera were isolated.

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674. THE LACTIC ACID AND VOLATILE FATTY ACID CONTENTS OF FIVE LOCALLY MADE TYPE CHEESES

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(With 3 Figures)

A study has been made of the lactic acid and lower volatile fatty acid contents of five locally made type cheeses, Coulommier, Gervais, Pont l'Évêque, Dutch and Smallholder. The Smallholder cheese is of the cheddar variety. All the cheeses were made from the same batch of milk on the same day, and the conditions of ripening of each type have been as closely akin as possible to those normally encountered in the areas peculiar to the individual cheeses. The changes which have been studied relate to those which occur between the time of manufacture and the normal time of placing the cheeses on the market. No attention has been paid to later maturing changes.

It might be reasonably expected that lactic acid, as a strong acid, would have a dominant effect on pH and titratable acidity. This is true in *in vitro* studies on the activity of hetero-fermentative strains of lactobacilli, but in milk, as noted by Pederson & Bagg (1), the buffering effect of milk constituents results in higher degrees of acidity than might be expected from the pH. These effects will be exaggerated in cheese. It may be noted here, in passing, that estimations of lactic acid are in fact estimations of lactate and that therefore titratable acidity and pH will not be directly related to the figures obtained for lactic acid. Troupe & Kobe (2) have in fact shown that the reaction which readily occurs between lactic acid, in the monomeric form, and methanol results in a fall of titratable acidity.

In general, the higher volatile fatty acids, caproic, caprylic and capric, seem to be characteristic of cheeses in which moulds play an integral part, such as Roquefort (Currie) (3). They seem to be characteristic also of the later stages of ripening of harder cheeses such as Cheddar (Suzuki, Hastings & Hart (4); Peterson & Johnson (5)). Acetic acid appears to be the most commonly occurring acid in the early stages of cheese ripening, but Suzuki *et al.* (4) noted the presence of formic acid although later references to its presence are not frequent. In the present work this latter acid has been encountered in all the fatty acid analyses performed. Formic acid occurs as a result of bacterial metabolism, for example in silage (Barnett) (6), but its occurrence in combined form in lipids has been noted only in recent years (Barnett & Duncan (7); McInnes, Hansen & Jessop (8)). It is not clear whether the formic acid in cheese results from bacterial metabolism or from lipid breakdown. Butyric acid, in the early stages of ripening, may result from the breakdown of lactose vial actic acid as Hillig & Montgomery (9) suggest may happen in cream. Again, Wolf (10,11) states that lactic streptococci and lactobacilli contain butyrinases and that it is possible that increases in butyric acid may be caused by butyrinase liberated from the dead cells of lactobacilli.

EXPERIMENTAL

Cheese. The cheese samples used in this work were obtained from the North of Scotland College of Agriculture (Craibstone Farm). The different cheeses were made at the same time from the same batch of milk and the starter used was a mixed strain type including acid-producing strains (three strains of *Streptococcus cremoris* and one strain of *Str. lactis*). In addition, the starter included two V.P. positive strains—one, an active acid producer (*Str. diacetilactis* type) and the other a non-acid producer.

As far as possible the cheeses were each made and stored under conditions suitable to their type, and except for Smallholder sufficient individual cheeses were made to enable one cheese to be used as a complete representative sample. Thus, four Coulommiers were made, four Gervais, five Pont l'Évêques, three Dutch cheeses and two large Smallholders. The Smallholders were sampled by means of a scoop at the necessary intervals.

The aim of the work was to study the changes that occur up to the point at which the cheeses would normally be marketed. Thus, the Coulommier was sampled for 3 days, the Gervais for 3 days, the Pont l'Évêque for 4 weeks, the Dutch for 2 weeks and the Smallholder for 7 weeks, the initial samples being removed and stored at the time of manufacture.

Each sample when removed from the maturing room was transported to the laboratory and kept in a deep-freeze at $-20°$ C. in an air-tight plastic bag till all the samples had been collected. During the different analyses the samples were never kept out of the deep freeze for longer periods than were absolutely necessary. All cheese analyses were performed on rind-free samples.

ANALYTICAL

Volatile fatty acids (V.F.A.). The acids were extracted from the cheese samples by the method of Barnett & Tawab (12). An amount of distillate from the Markham, equivalent to between 0·5 ml. and 1·0 ml. of N/50NaOH, was used for each V.F.A. estimation by gas chromatography, using the technique of James & Martin (13). While the procedure adopted was similar in all essentials to that of these latter authors, the recoveries of V.F.A. were not as good as had been hoped for. After considerable experimentation, it was found that excellent recoveries could be obtained by incorporating the following three slight modifications, viz.:

(1) Before evaporating the above neutralized distillate to dryness, 0·1 ml. 2 N-NaOH should be added to prevent loss of formic and acetic acids.

(2) The micro-column used had a total length of 17 cm. and was made from tubing 4 mm. in diameter. The column was slightly flared at one end and sealed at the other. The aperture was situated on the side of the tube about 3 mm. from the closed end and the tube was packed first with a 5 mm. thick wad of fibreglass, followed by 2 cm. of anhydrous Na_2SO_4 and lastly by 2 cm. of Celite.

(3) The free V.F.A. were transferred to the column and washed through it with a total of 1·8 ml. of ether. The variation in results obtainable by the use of the normal micro-column and the slightly modified one are indicated by the following trials.

Trial A. (1) A standard mixture of volatile fatty acids from C_1 to C_{10} was prepared in ethereal solution. An aliquot of this solution was shaken with 2 N-NaOH solution, the ether removed by evaporation and the extract distilled, after acidification, from the Markham to obtain the titration value.

(2) An aliquot similar to that used in (1), was loaded directly on to the micro-column, and 1·8 ml. anhydrous ether were used for washing through the column. The acid filtrate was collected in 2 N-NaOH and distilled from the Markham in the usual fashion, after removal of the ether by evaporation. The titration value was compared with that obtained in (1) above. The acid recoveries were 98–100 % in both cases.

Trial B. Step (1) in Trial A was repeated, but the neutralized distillate was treated according to the procedure described above as being used for V.F.A. determinations on cheese. The acid filtrate from the micro-column was again collected in 2 N-NaOH, the ether evaporated and the acids removed by distillation from the Markham.

Trial C. Trial B was repeated, but a cheese extract of known titration value was used instead of the standard ethereal solution of V.F.A.

Each of these trials was prepared in duplicate. One of the duplicates was put through a standard James and Martin micro-column, while the other was put through the modified column. The results are shown in Table 1. It is clear that the modified column gave a greatly improved recovery with B and C. It was found that the improvement was attained in part by the modified column itself, but maximum recovery was obtained by combining the modifications. It is considered that, since equivalent results were obtained with repetitions of the different trials, the identical recoveries in Trial A were due to the absence of small amounts of phosphoric acid which were present in Trials B and C. It is conceivable that the phosphoric acid has a retarding effect on the passage of the ether solution, under air pressure, down the column.

Table 1. *The recoveries of total volatile fatty acids directly from a standard ethereal solution (A_2), from a standard ethereal solution subjected to normal pre-treatment before being loaded on to the micro-column (B) and from a cheese extract (C) treated similarly to B (see text)*

	Recovery of volatile fatty acids (%)			
	Standard micro-column		Suggested micro-column	
Trial no.	(1)	(2)	(1)	(2)
A_2	100	100	100	100
B	80	82	98	98
C	80	80	97	98

Good separations have been obtained, where both lower and higher V.F.A. were involved, by using two different vapour sources, one steam, the other ethylene glycol mono-ethyl ether, for the vapour jacket. This is in accord with the findings of Van der Kamer, Gerritsma & Wansink (14) who used a special thermostatically controlled heating arrangement for the outer jacket. It may be noted that the quantities of formic acid with which we have been involved in cheese have been such that they have not been lost during the transfer of the free V.F.A. to the micro-column by suction.

Lactic acid. Lactic acid has been determined in cheese in various ways, but in the present work it was decided to use a method incorporating the colorimetric technique of Barker & Summerson (15), chiefly because the method has been used in these laboratories, in modified form, in studies on the lactic acid content of silage (Barnett (16)). Kleinert (17) used the method, adapted from that described by Davidson (18), for milk. The following procedure, based on the work of these authors, has been used here and found to give satisfactory

results, and its efficiency has been confirmed by recovery tests with mixtures of fresh cheese and standard lactate.

Of the cheese sample, 1 g. is minced, in the case of hard cheese, and placed in a Pyrex test-tube (200 mm. × 25 mm.) with 30 ml. N/4 NaOH. If the cheese is a soft one, the sample is placed in the tube and blended, using a spatula, with the NaOH. The tube and its contents are kept in a water-bath at 70° C. for 1 hr. with occasional shaking. While still hot the contents of the tube are transferred quantitatively to a 100 ml. standard flask and the volume made up to the mark after cooling. A 1 ml. portion is taken in a 50 ml. standard flask and diluted to about 35 ml. with water. The procedure thereafter is similar to that described by Davidson (18).

After development of the colour with the *p*-hydroxy-diphenyl reagent, the reading is made on an EEL colorimeter using a green filter. In calculating the lactic acid content of the cheese samples, volume correction factors, as used by Davidson for milk, were used for cheese because the protein and fat content of 10 ml. of milk approximates to the amounts of these components present in 1 g. of cheese while the amounts of $Ca(OH)_2$ suspension and $CuSO_4$ solution were similar in both. In practice, six samples were examined at the same time along with a standard (lithium lactate) and a blank.

RESULTS

The pH changes during the ripening periods of the different cheeses are shown in Fig. 1, while the total titratable acidity changes in the fresh cheese are indicated in Fig. 2. It is clear that, considering the results for all the samples analysed, there was no close correlation between pH and total titratable acidity, but such an overall correlation could hardly

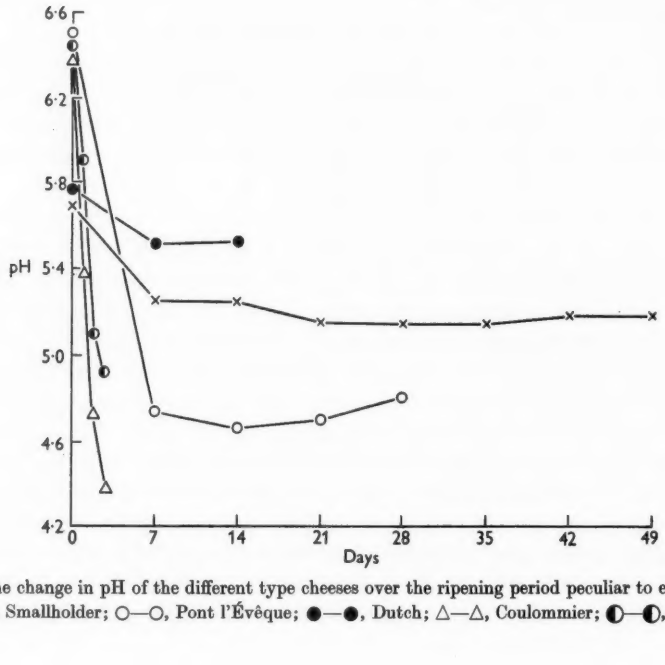

Fig. 1. The change in pH of the different type cheeses over the ripening period peculiar to each cheese. ×—×, Smallholder; ○—○, Pont l'Évêque; ●—●, Dutch; △—△, Coulommier; ◐—◐, Gervais.

be expected since so many factors govern titratable acidity in cheese. For each particular cheese, however, the relationship between pH and titratable acidity is obvious (Fig. 3).

Changes in the total V.F.A. content of the cheese are indicated in Table 2. These results have to be considered in the light of the fact that the terms 'volatility in steam', 'water solubility' and 'fat solubility' as applied to fatty acids are only relative. Thus in the present work the quantities of cheese extract used in the Markham were such that any

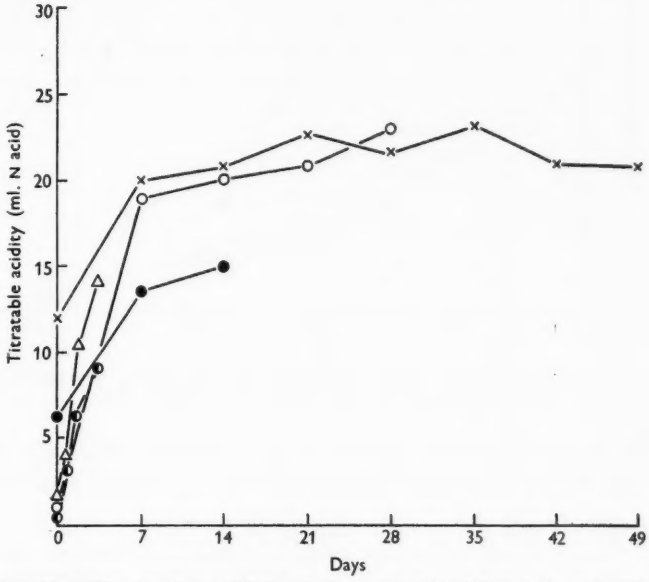

Fig. 2. The titratable acidity (in ml. N acid/100 g. fresh cheese) of the different cheese samples throughout the ripening period peculiar to each cheese. The symbols used correspond to those in Fig. 1.

acids from C_1 to C_9, in amount likely to be encountered, would have been soluble in the volume of aqueous distillate collected. Again, it is not always realized that even palmitic acid is slightly volatile in steam while myristic acid will distil from a Markham at the rate of about 1 mg./80 ml. of distillate under normal conditions of operation. These remarks bring into prominence the amounts of water insoluble fatty acids which were obtained from the different samples examined. The amounts varied from 16·3 to 50·0 % of the total acids distilled as measured by titration values. A preliminary suspicion would be attached to the extraction of the ether solution of fatty acids with sodium hydroxide employing the mild extraction method used by Smiley, Kosikowsky & Dahlberg (19) and by Barnett & Tawab (12), but, as one of the tests of the method, various triglycerides of higher and lower fatty acids were subjected, in ethereal solution, to the complete extraction technique and gave rise to no free fatty acid production. Again, it will be seen from Table 2 that for each cheese analysed, under identical conditions of extraction, there was a steady increase or decrease in the amounts of this insoluble fraction. Runs with different samples of the water-insoluble fraction on the gas chromatographic column indicated minute traces of caproic acid (*c.* 0·0006 % of the dry matter of the sample of cheese involved), but no other acid, although the runs were continued under high nitrogen

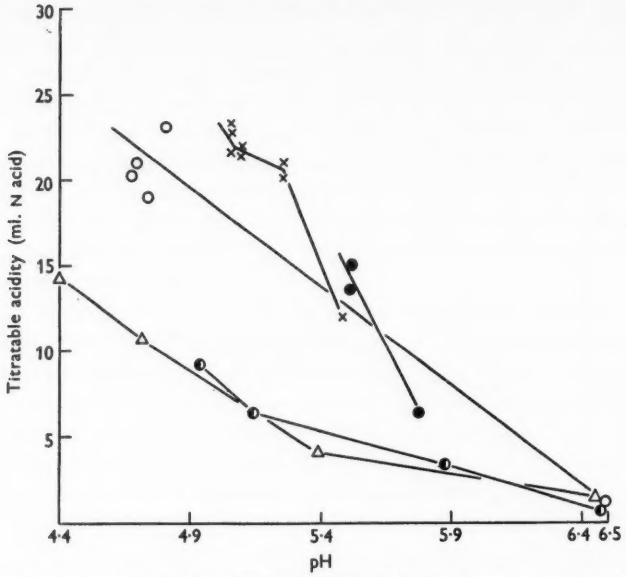

Fig. 3. The relation between titratable acidity and pH for each type of cheese over the ripening periods considered. The symbols used correspond to those in Fig. 1.

Table 2. *The volatile fatty acids, expressed as ml.* N/10 *alkali, obtained by distillation of extracts of the various cheese types at different stages of maturity*

Type	Age	Volatile acidity (≡ml. N/10 alkali) in 100 g. fresh cheese: Water soluble	Water insoluble	Total	Water insoluble volatile acids (% of total volatile acids)
		Soft cheese			
Coulommier	Start	3·8	3·8	7·6	50·0
	1 day	3·5	1·3	4·8	27·1
	2 days	11·1	2·6	13·7	19·0
	3 days	13·2	2·6	15·8	16·5
Gervais	Start	3·1	1·6	4·7	34·0
	1 day	4·2	2·6	6·8	38·2
	2 days	9·5	3·2	12·7	24·4
	3 days	11·1	4·7	15·8	29·8
Pont l'Évêque	Start	5·2	2·1	7·3	28·8
	1 week	9·5	4·2	13·7	30·6
	2 weeks	9·5	4·2	13·7	30·6
	3 weeks	9·0	5·8	14·8	39·2
	4 weeks	9·5	9·5	19·0	50·0
		Hard cheese			
Dutch	Start	11·6	2·6	14·2	18·3
	1 week	13·7	4·2	17·9	23·5
	2 weeks	17·0	4·2	21·2	19·8
Smallholder	Start	11·0	2·6	13·6	19·1
	1 week	15·8	3·7	19·5	19·0
	2 weeks	17·0	3·7	20·7	17·9
	3 weeks	19·0	3·7	22·7	16·3
	4 weeks	20·0	5·2	25·2	20·7
	5 weeks	20·0	5·3	25·3	20·9
	6 weeks	21·1	6·3	27·4	23·0
	7 weeks	21·1	6·3	27·4	23·0

pressure for 60 hr. which would have been sufficient to bring out lauric and perhaps myristic acids. Moreover, the fractions recrystallized from aqueous alcohol melted over the range 47°–51°. Presumably, then, higher acids are involved, but it is significant that oleic acid, which is present in greatest abundance in the triglycerides of milk fat, was not present. The acid is somewhat volatile in steam and a trace of it would have resolved the solid obtained into an oil. It must be assumed, therefore, that these acids are produced by selective lipolysis of the triglycerides. The problem is being further investigated.

Table 3. *The individual volatile fatty acids, expressed as mg./100 g. fresh cheese, obtained by gas chromatography of the distillates from the various cheese types at different stages of maturity*

Type	Age	Volatile acids (mg./100 g. fresh cheese) Formic	Acetic	Butyric	Total
		Soft cheese			
Coulommier	Start	4·7	13·0	5·6	23·3
	1 day	5·0	19·1	0·0	24·1
	2 days	6·5	57·3	0·0	63·8
	3 days	7·7	65·7	0·0	73·4
Gervais	Start	3·7	13·1	0·0	16·8
	1 day	2·9	16·4	1·9*	21·2
	2 days	9·0	35·4	0·0	44·4
	3 days	7·7	53·1	0·0	60·8
Pont l'Évêque	Start	4·8	18·7	7·3	30·8
	1 week	5·8	37·9	0·0	43·7
	2 weeks	7·8	33·9	2·5	44·2
	3 weeks	13·6	25·4	7·5	46·5
	4 weeks	12·9	27·0	9·9	49·8
		Hard cheese			
Dutch	Start	5·8	50·6	0·0	56·4
	1 week	15·5	50·6	0·0	66·1
	2 weeks	18·2	57·6	10·0	85·8
Smallholder	Start	11·6	52·7	0·0	64·3
	1 week	5·8	73·3	3·7	82·8
	2 weeks	7·8	73·8	7·5	89·1
	3 weeks	9·7	75·8	11·2	96·7
	4 weeks	15·4	75·4	14·8	105·6
	5 weeks	19·4	80·9	17·4	117·7
	6 weeks	15·5	85·9	22·3	123·7
	7 weeks	15·5	85·9	22·3	123·7

* Iso-butyric.

The results for the individual water-soluble V.F.A. contents of the different cheese samples are indicated in Table 3. It is immediately clear that acetic acid was the most abundant, while formic acid was a much commoner constituent than butyric acid. Indeed, except at the start of the ripening process with Coulommier, butyric acid did not appear at all in this cheese while only a trace of iso-butyric acid was found on the second day of ripening of Gervais. Pont l'Évêque and Smallholder cheeses, however, contained considerable amounts of butyric acid in the later stages of the ripening period. Despite the running of the chromatograms to exhaustion no other V.F.A. were detected. When it is remembered that these results are calculated on the fresh cheese it is very obvious that the production of the acids was very much greater in the soft than in the hard cheese; this of course would be expected owing to the accelerating effect of moisture on ripening. The presence and subsequent disappearance of butyric acid at the start of the ripening period

in Coulommier is quite characteristic of fermentations in which fatty acids are involved. The same remarks apply to the iso-butyric acid present on the first day of the ripening of the Gervais and to the butyric acid at the start of the ripening of the Pont l'Évêque. These acids may form metabolic substrates for different organisms at different pH levels. The

Table 4. *The lactic acid content of each cheese at different stages of ripening, expressed as a percentage of the fresh cheese*

(Only the samples relevant to each ripening period were analysed, see text.)

			Lactic acid content (% of fresh cheese)									
			Days			Weeks						
Type	Cheese	Start	1	2	3	1	2	3	4	5	6	7
Soft	Coulommier	0·883	1·325	1·457	1·811	—	—	—	—	—	—	—
	Gervais	0·883	1·060	1·264	1·345	—	—	—	—	—	—	—
	Pont l'Évêque	0·856	—	—	—	1·916	1·590	1·101	1·060	—	—	—
Hard	Dutch	0·937	—	—	—	1·508	1·345	—	—	—	—	—
	Smallholder	0·954	—	—	—	1·590	1·391	1·073	1·590	1·073	1·550	1·351

results for the lactic acid content of the different samples are given in Table 4. With the soft cheese over the short ripening period studied the lactic acid increased from about 0·9% (fresh material) to about 1·8% in the Coulommier and to about 1·3% in the Gervais. The figures for the hard cheeses were in the main higher than those for the soft cheeses as might be expected because of the longer ripening period. All of them were much higher after one week of ripening than they were at the start of the process, but some lower values were subsequently obtained, presumably due to metabolism of the lactic acid by the cheese microflora.

DISCUSSION

The work reported here has, with particular regard to lactic acid, served to confirm much of what has been done at other centres. The formation, in the early stages of ripening, with a subsequent complete or partial disappearance of this constituent has been noted by other workers (e.g. Hurel & Mocquot[20]), while the later fluctuations in the lactic acid content of Smallholder cheese are somewhat similar to those noted by Suzuki *et al.*[4] in Cheddar cheese. It might appear permissible to suggest that some of the starter organisms are hetero-fermentative in nature and that variations in lactic acid content should be paralleled by corresponding variations in acetic acid content. This suggestion is not apparently tenable, however, because the acetic acid figures for Smallholder cheese (Table 3) showed no such fluctuation. One can but assume that, in the ripening process, the lactic acid initially formed acts as a substrate for some organism or organisms and that its later formation and disappearance are due to the breakdown and utilization of components other than lactose such as amino acids and protein. For example, the hydrolytic deamination of alanine would give rise to lactic acid.

The lack of variety of fatty acids noted in these early stages of cheese ripening suggests that lipolytic and deamination effects were not greatly in evidence. Nevertheless, the high percentage of higher volatile fatty acids, that is acids higher than caproic, is of interest and not readily explicable. Although the synthesis of higher fatty acids by microorganisms has been studied (e.g. by Bornstein & Barker)[21], the synthesis of acids higher than caproic from lower V.F.A. has not been noted, as far as the authors are

aware. That being so, one is led to the conclusion that very small amounts of higher fatty acids are readily broken off from some lipid constituent in cheese. It would appear that there is here a field for further investigation.

The occurrence of formic acid in such quantity as has been noted makes it surprising that more attention has not been paid in the past to its possible significance. Doubtless this is due to the difficulty of identifying and determining this substance except by the chromatographic methods that are now available.

SUMMARY

1. A study has been made of the development of titratable acidity, lactic acid and volatile fatty acids in five locally made type cheeses. The study of the changes involved has been made over the period of ripening up to the stage at which each type would normally be marketed. All the cheeses were made from the same batch of milk at the same time.

2. Slight modifications to the micro-column devised by James & Martin (13), and its use, have been suggested which have resulted in improved recovery of volatile fatty acids.

3. The volatile fatty acids have been determined *in toto* and also as separate constituents. Formic acid has been noticed as a common constituent of the acid mixture in all the cheeses, while, over the ripening period involved and apart from unidentified acids higher than caproic, the only other volatile acids encountered have been acetic and butyric, and on one occasion iso-butyric.

4. Over the short ripening period studied, the lactic acid content of the soft cheeses rose, but after the initial rise in Pont l'Évêque and in Dutch cheese the results showed a downward trend. Fluctuations in the lactic acid content of the Smallholder cheese have been noted which compare with the corresponding findings of other workers.

5. All the results presented have been calculated on the basis of fresh cheese because this is the customary practice and comparisons with other work would be difficult if this had not been adhered to.

The authors are greatly indebted to Miss Dickson of the Dairying Staff of the North of Scotland College of Agriculture (Craibstone Farm), whose help and advice in the preparation of the cheese samples have been invaluable.

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675. THE CARBON DIOXIDE CONTENT OF NEW ZEALAND CHEDDAR CHEESE

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(With 4 Figures)

INTRODUCTION

The carbon dioxide content of maturing Cheddar cheese has not been adequately investigated, though the importance of its role in the formation of slit openness has often been suggested. The presence of numerous slits was considered to be a serious defect in New Zealand Cheddar cheese, until the use of starters consisting of single strains of *Streptococcus cremoris* and *Str. lactis* became widespread in the middle 1930's. The improved closeness which resulted was attributed to the absence of gas-producing betacocci, which were known to be present in most commercial mixed starter cultures formerly used as starters. Mixed starter cultures may also contain strains of *Str. diacetilactis* which are gas-producing (Czulak (1)).

In the present investigation, the contrast between the carbon dioxide content of cheese made from pasteurized milk with single strain and mixed starters has been found to be very clear-cut and to be associated with marked differences in openness of texture. The negligible production of carbon dioxide by the single strain starters themselves has made it possible to study the carbon dioxide formed by the natural flora of cheese made with these starters.

Clark (2), Koestler & Hostettler (3) and Hostettler (4) estimated the carbon dioxide in Emmental cheese by drawing the gas off under vacuum, whilst Hiscox, Harrison & Wolf (5) estimated the dissolved carbon dioxide of Cheddar cheese in connexion with the estimation of volatile acids by subtraction of the values obtained with aerated samples from the values obtained with non-aerated samples. Swartling & Willart (6), who worked with Herrgård cheese, emulsified their sample in alkali, acidified and entrained the carbon dioxide in carbon dioxide-free air from which it was absorbed in a standard solution of barium hydroxide. The present method, which was derived independently, was similar to that of Swartling & Willart, except that the cheese sample was emulsified in distilled water, not alkali, and was not acidified except when the cheese was less than one week old.

EXPERIMENTAL

Cheese investigated. The carbon dioxide content of twenty-seven Cheddar cheeses (mostly 80 lb. 'exports'), made from good quality pasteurized milk at the dairy factory of this institute, was investigated at intervals after manufacture. Of these cheeses, twenty-one were made with the use of single strain starters and six with the use of commercial mixed starters, which contained heterofermentative strains of lactic streptococci as evidenced by an aroma of diacetyl and the appearance of gas bubbles when the coagulated starter was warmed.

Sampling. Samples were obtained by inserting a large butter trier into the cheese from its upper flat surface and withdrawing a plug of cheese about two-thirds the height of the cheese in length. A sample of 30–40 g. was taken from the lower end of the plug. Thus, only cheese at a considerable distance from the flat surfaces was sampled, the object being to avoid taking cheese from near the surface which might have a lower carbon dioxide content as a result of gas diffusion into the atmosphere. In this connexion, the distance of the plug radially from the centre of the flat surface also has a bearing, since gas also diffuses from the sides of the cheese. This point will be referred to later.

The sample was transferred rapidly to a weighed Waring blendor jar containing 100 ml. of freshly boiled distilled water. The jar was immediately sealed with a lid and re-weighed.

To refill the sample hole in the cheese, the unused portion of the plug plus sufficient other cheese of a similar age was grated and rammed into the hole and the top sealed with molten wax. Subsequent samples were usually drawn at the same distance from the centre, but at least 1 in. removed from the site of the previous sample.

Estimation of carbon dioxide. Emulsification of the sample in the sealed blendor was followed by acidification to pH 3–4, with 5 ml. of 2 N sulphuric acid, when the sample was milk, curd or cheese, less than 1 week old. For 1 hr. carbon dioxide-free air was bubbled through the emulsion which was intermittently re-blended (7 sec. every 1½ min.). Carbon dioxide entrained in the air was absorbed in a Pettenkofer type absorption tube containing 20 ml. of a standard (0·150–0·200 N) barium hydroxide solution. The flow of gas through the system was adjusted to 40–100 c.c./min., the bubbles in the absorption tube having 7–10 sec. in contact with the alkali.

The barium hydroxide which had been partly neutralized by the carbon dioxide from the cheese was back-titrated to a phenolphthalein end-point with a standard solution of oxalic acid. After 'blank' correction the quantities of carbon dioxide absorbed were expressed as millilitres (Standard Temperature and Pressure) per gram (ml./g.) of cheese (1 ml. of carbon dioxide at S.T.P. weighs 1·96 mg.).

RESULTS

Carbon dioxide concentration gradient and reproducibility of results

In the development of the technique for the estimation of carbon dioxide, it was definitely established that the carbon dioxide concentration decreases towards the outer surfaces. The existence of a carbon dioxide gradient is demonstrated in Fig. 1, which shows that the concentration gradient exists to a depth of several inches, thus indicating an appreciable diffusion rate (greater than the rate of diffusion of moisture in the cheese (Scott (7))). Swartling & Willart (6), working with Herrgård cheese, have demonstrated a higher concentration of carbon dioxide towards the centre of this type of cheese, but they interpret this as being due to the effect of a more gradual drop in temperature and rise in salt content on bacterial activity in the inner portions of the cheese. These factors are unlikely to exert an appreciable influence in Cheddar cheese.

Where the influence of age of cheese on the carbon dioxide content is being followed, the existence of a gradient makes it important that all plugs should be drawn at a constant distance from the centre. Under these conditions there is good reproducibility of the quantities of carbon dioxide found even when the samples are taken in different parts of a cheese (see Table 1) or from different cheeses from the same vat (see Table 2).

The reproducibility of the values obtained tends to decrease with increasing maturity of the cheese. This variability may be related to an increasingly open texture which may increase the carbon dioxide losses during sampling and alter the distribution of carbon dioxide within the cheese.

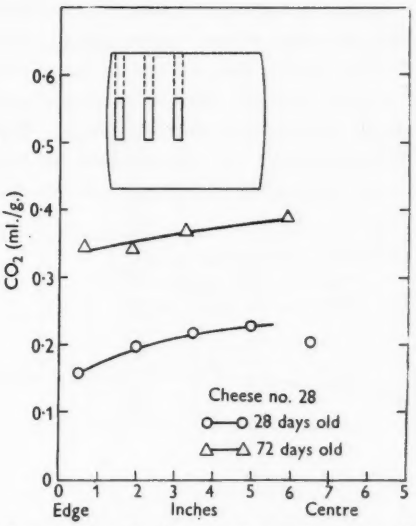

Fig. 1. Concentration of carbon dioxide at various distances from the curved surface.

Table 1. *Typical reproducibility of the concentrations of carbon dioxide found in cheese*

	Cheese 1A (single strain starter)			Cheese 10 (mixed starter)	
Age (days)...	5	12	19	86	87
CO_2 (ml./g.)	0·077	0·129	0·225	0·372	0·392
	0·077	0·123	0·219	0·417	0·397

All samples were drawn 4½ in. from the centre.

Table 2. *Concentrations of carbon dioxide in cheese from the same vat*

Single strain starter (KH and R_1)			Mixed starter		
Cheese no.	Age (days)	Carbon dioxide (ml./g.)	Cheese no.	Age (days)	Carbon dioxide (ml./g.)
7	22	0·206	10	14	0·360
8	20	0·207	10	27	0·388
9	20	0·186	11	20	0·358
			12	20	0·361
			13	20	0·385

All cheeses were manufactured 9 March 1954. All plugs were drawn 4½ in. from the centre.

Influence of starter type

The quantities of carbon dioxide found in Cheddar cheese made with single strain starters and with commercial mixed starters, are radically different until at least a month from manufacture. Cheese made with mixed starter is characterized by a rapid rise in the carbon dioxide content in the days immediately following manufacture. Within 10–20 days

the rise in carbon dioxide concentration ceases and there is little further change in the next 6 months.

Single strain starter cheese is characterized by little or no change in the carbon dioxide content in the first 5–12 days from manufacture. After this initial quiescent period the carbon dioxide levels may follow one of two courses. In the first and less common case, the carbon dioxide content may remain almost unchanged, or rise only slightly during the maturing of the cheese. In the second case, typical of most of the single strain starter cheese investigated, the carbon dioxide content rises gradually until the cheese is 2–3 months old, after which there is little further change. This static upper level of carbon dioxide concentration is similar to that attained by a mixed starter cheese in 10–20 days, and appears to be almost the same from cheese to cheese.

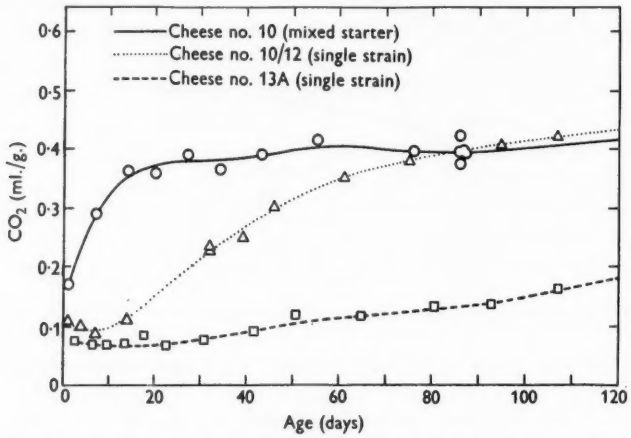

Fig. 2. Curves showing the carbon dioxide found at varying ages in mixed starter and single strain starter cheeses.

The initial difference between the single strain and mixed starter cheese is demonstrated clearly in Fig. 2 (and Fig. 4), which also shows the typical difference between the two types of carbon dioxide formation in single strain starter cheese.

Again, the two classes into which the carbon dioxide accumulation in single strain starter cheese may fall is demonstrated in Fig. 3, in which the carbon dioxide content of cheese may be seen to be either less than 0·2 ml./g. or more than 0·3 ml./g. From the figure it is apparent that the quantities of gas which accumulate in cheese may differ radically from the beginning to the end of a dairying season.

In New Zealand all cheese for export is moved to the coastal grading stores, and graded about 14 days after manufacture. At this time the difference in carbon dioxide content between single strain and mixed starter cheeses is about at a maximum, and is reflected in the difference in openness of texture when graded. At maturity the concentration of carbon dioxide found in mixed starter cheeses, and in many single strain starter cheeses is approximately the same, but the more gradual accumulation in single strain cheeses results in an appreciably closer texture than is found in mixed starter cheeses. Those single strain starter cheeses in which virtually no carbon dioxide accumulates show a remarkably close texture at maturity.

Temperature of storage

A preliminary investigation of the influence of storage temperature on the carbon dioxide content of cheese was made with four cheeses; two manufactured using single strain starters and two using mixed starters.

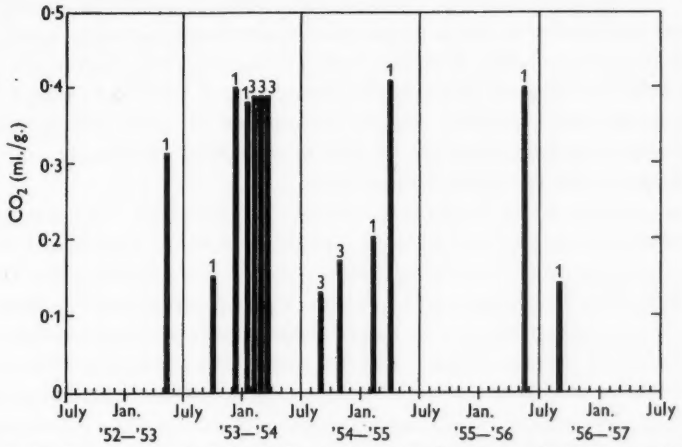

Fig. 3. The carbon dioxide content of the single strain starter cheeses examined. The numbers above the columns indicate the number of cheese represented by each column. The height of the columns represents the carbon dioxide content of cheese approximately 3 months old (time of little change).

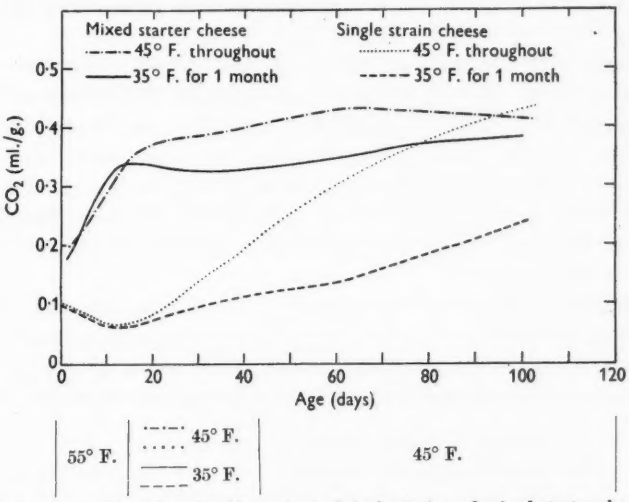

Fig. 4. The influence on the carbon dioxide content of single strain and mixed starter cheeses of storage at 35° F. or 45° F. for 1 month with subsequent storage at 45° F.

After the usual 14 days at 55° F. one of the single strain and one of the mixed starter cheeses were transferred to storage at 35° F., and the other two cheeses were transferred to storage at the normal 45° F. After one month at 35° F., the storage temperature of the first pair of cheeses was raised to 45° F. As may be seen from Fig. 4, the rise in carbon

dioxide within both the single strain and mixed starter cheeses was appreciably retarded when stored at the lower temperature. This retarding influence appeared to persist even after the storage temperature was raised to normal.

DISCUSSION

In the first week after manufacture the absence of carbon dioxide formation in pasteurized milk cheeses made with the use of single strain starters, and the rapid accumulation of carbon dioxide in cheeses made from the same milk but with the use of mixed starters, is associated with the difference in the bacterial composition of these two types of starter. The single strain starters contain only *Str. cremoris* or *Str. lactis* strains which are incapable of carbon dioxide formation, whereas mixed starters frequently contain carbon dioxide-producing betacocci and *Str. diacetilactis*.

The carbon dioxide, which begins to accumulate in many single strain starter cheeses a week or two after manufacture, is probably a result of the rise to dominance of lactobacilli in place of the dying streptococci of the starter. Sherwood (8) has shown that many lactobacilli, including the 'homofermentative' strains, form appreciable carbon dioxide in milk culture, and he has also shown by inoculation of cheese milk with pure cultures that some types of lactobacilli produce an open texture in cheese. Since infection with lactobacilli is normally a matter of chance, the types which occur in cheese may vary from place to place and from time to time. The immediate source of these organisms is not known, but it has been suggested that their habitat is the pasture grasses and clovers. If this is so, the type of plant, climate, and weather conditions could determine the types of organism occurring in cheese. The results indicate that many cheeses become infected with gas-producing types, while in others the flora is dominated by types which do not produce carbon dioxide.

Storage temperature may also affect the ratios of different bacterial types which develop within a cheese. The results of the experiment on the influence of the storage temperature suggest that a lower temperature suppresses carbon dioxide formation, and that the effect persists for a considerable time after the temperature is again restored to normal. The suppression of carbon dioxide production may be due to reduced bacterial metabolic activity at the lower temperature, while the persistence of the effect suggests that the normal flora has been replaced by a more psychrophilic flora which contains fewer carbon dioxide producers.

The effect of gas formation on cheese texture depends on several factors. Where the gas is produced immediately after manufacture by betacocci from a mixed starter, the cheese invariably has an open texture upon examination at 2–3 weeks. Where gas is produced after 2–3 weeks by lactobacilli the effect on texture depends amongst other things upon:

(1) The total volume of gas produced.
(2) The rate of production.
(3) The consistency of the cheese body.
(4) The mechanical treatment of the cheese.

If the carbon dioxide can diffuse out of the cheese quickly enough there may only be a minimum effect by way of gas-filled cracks in the cheese body. If the carbon dioxide is formed too quickly for diffusion, or the cheese is handled roughly, the separation of a gas phase may occur giving rise to many slits and cracks. The actual mechanics of the forma-

tion of an open texture in Cheddar cheese is probably largely analogous to the formation of 'eyes' in the more plastic body of Swiss-type cheese which has been investigated by Clark (9) and Hostettler (4).

The method used measures the carbon dioxide in solution, and except where small slits are enclosed within the plug, the carbon dioxide in the gas phase will not influence the values obtained. It is thus possible that the upper limit of about 0·4 ml. carbon dioxide per gram found in both mixed starter and many single strain starter cheese, represents the limit above which this gas cannot remain in solution. Other possible interpretations of the significance of the upper level may be that the rate of formation of carbon dioxide is equalled by the rate of loss, or that no further gas is formed in the absence of a suitable substrate. In this latter connexion, Swartling & Mattsson (10) have shown that the carbon dioxide content of Herrgård cheese reaches a maximum at the time of complete utilization of citrate.

SUMMARY

Some of the factors influencing the concentration of carbon dioxide found in New Zealand Cheddar cheese have been investigated.

1. Cheeses made with the use of commercial starters (containing betacocci) are characterized by a rapid increase in their carbon dioxide content during the 2 weeks following manufacture.

2. Cheeses made with the use of single strain starters do not change in carbon dioxide content in the first 2 weeks following manufacture, but may ultimately contain as much carbon dioxide as commercial starter cheeses.

3. High concentrations of carbon dioxide within a cheese result in an open texture, especially when the carbon dioxide is formed shortly after manufacture.

4. The loss of carbon dioxide to the atmosphere is demonstrated by the existence of a carbon dioxide concentration gradient within the cheese.

5. Storage of cheese at a lower temperature than is usual results in retarded carbon dioxide formation.

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676. DIFFUSE REFLEXION SPECTRA OF DAIRY PRODUCTS IN THE NEAR INFRA-RED REGION

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(With 4 Figures)

INTRODUCTION

In the visible region of the spectrum much of the radiation incident upon milk and butter surfaces is scattered, and can be examined by a study of the diffuse reflexion of these materials. Burgess & Herrington (1) have shown that more than half the visible radiation falling on a 1 cm. layer of whole milk is reflected, and that the reflectance increases at longer wavelengths to more than 70% in the yellow region of the spectrum (0·58μ). Homogenized milk reflects even more radiation than untreated milk of the same fat content. Lagoni and Merten (2) have studied the diffuse reflexion of milk in the region 0·36μ–0·70μ and have discussed the effects of homogenization and dilution. Burton (3) has shown how reflectance measurements made in the visible region with a simple reflectometer can provide useful information about the structure of milk and also the effects of heat treatment. Mohr and his co-workers have studied in detail the colour of butter (4) and also of dried milk powders and condensed milk, with particular reference to the colour of the coffee made from the milk.

The globule and particle size of dairy products plays an important part in determining the optical properties of these materials. In regions where the particle sizes are of the same order as the wavelengths of the radiation, large changes in optical properties are usually observed. Liquid milk and butter have globule diameters of the order of microns, corresponding to wavelengths in the near and fundamental infra-red regions (0·5–15μ). It would therefore be expected that when the globule sizes are altered by homogenization of the milk, changes in the optical properties would show in this region of the spectrum. Globule size and distribution are also major factors governing the physical properties of butter, and some correlation between optical properties and the structure of butter is therefore expected.

The infra-red absorption spectra of dairy products in the 2–15μ region has already been investigated (5) and the information presented here covers the region between the visible spectrum and 2·5μ. In the visible region the reflexion method seems to be a useful technique for the study of dairy products, but at longer wavelengths the scattered radiation becomes very much less, and spectra can more easily be obtained by transmission techniques. In the near infra-red region it has been possible to obtain both transmission and reflexion spectra and a comparison will be made between the two methods.

Measurements of diffuse reflexion in the near infra-red region have so far been confined mainly to paints (6) and to human skin (7, 8, 9), but a preliminary communication by Goulden (10) indicates a wider application in the study of powders. Jacquez and others

have discussed the design of instruments for measuring diffuse reflexion in the near infra-red region (11). Although their results show that the comparison type of integrating sphere is preferable to the substitution type, the need to examine liquids and powders made it necessary to use a substitution type of reflectometer for work with dairy products.

EXPERIMENTAL

The transmission measurements reported here were made with a Grubb Parsons S3A Double-Beam Spectrometer fitted with a fused silica prism. Fused silica windows were used for the absorption cells; the path lengths of the 42 and 135μ cells being calculated from measurements of the wavelengths of the interference fringes obtained from the transmission of the empty cells. Some of the reflectance measurements were obtained with this spectrometer used as a single-beam instrument, but most of the measurements were made with a Leiss Double Monochromator fitted with fused silica prisms. A 24 V. 60 W. car lamp was used as a radiation source, power being supplied by a step-down mains transformer operated from a constant voltage supply. The radiation beam was interrupted at a frequency of 800 c.p.s. by a rotating sector wheel.

Spectrometer wavelength scales were calibrated using the emission spectrum of a mercury arc, and also by measurements of atmospheric water and carbon dioxide absorption bands. Wavelengths quoted for sharp bands are considered to be accurate to $\pm 0{\cdot}01\mu$.

Dispersed radiation from the monochromator was focused into the integrating sphere and formed a slightly enlarged image of the spectrometer exit slit on the sample surface. Diffusely reflected radiation from the sample was trapped inside the sphere and was detected by a Mullard lead sulphide photo-conductive cell (*A*), Fig. 1, fitted into the wall of the sphere. The rapid fall-off in sensitivity of this detector at wavelengths greater than $2{\cdot}5\mu$, together with strong absorption by the prisms, set an upper wavelength limit of $2{\cdot}5\mu$ to this investigation. An 800 c.p.s. tuned amplifier built according to the design of Brown (12) was used to amplify the signal from the detector. For normal operation the spectrometer was used as a non-recording instrument, using a smoked magnesium oxide surface as a standard.

In order to restrict observations to exactly the same part of the sample at all wavelengths, the slit-widths were kept constant and the output adjusted by changing the gain of the amplifier and the potential applied to the detector. For most of the measurements with the Leiss Double Monochromator, instrumental slit-widths of 0·15 mm. were used. This corresponded to spectral slit-widths of the order of $0{\cdot}01\mu$ and allowed the sample surface to be illuminated with a rectangular image $18 \times 0{\cdot}27$ mm.

The 4 in. diameter sphere was machined as two separate hemispheres from the aluminium block (*B*) (Fig. 1), and after polishing was coated on the inside surface with a layer of smoked magnesium oxide (*C*) about 1·5 mm. thick, according to the method of Dimitroff & Swanson (13). An aperture $\frac{3}{4}$ in. diameter at the top of the sphere allowed the radiation beam to enter, and a hole of the same diameter at the bottom of the sphere allowed the radiation beam to fall on to the surface (*D*) or on the magnesium oxide standard (*E*), according to the position of the sliding carriage (*F*).

In this design of reflectometer, the specularly reflected radiation follows the same path as the incoming beam and escapes from the sphere. The effects of specular reflexion were

tested by comparing the reflectance of a surface-aluminized mirror with that of the magnesium oxide standard. By this means it was possible to show that the specular reflexion component was less than $\frac{1}{2}\%$. By measuring the reflexion from a blackened cup, the effects of radiation entering the sphere by paths other than that of the beam from the monochromator were found to be less than $\frac{1}{4}\%$ at all wavelengths.

Liquid samples were examined in stainless steel cups 4 cm. deep. Owing to meniscus effects the liquid surface was not perfectly flat, and this would be expected to give rise to small errors in the general level of reflectance, but not to affect the general shape of the curves.

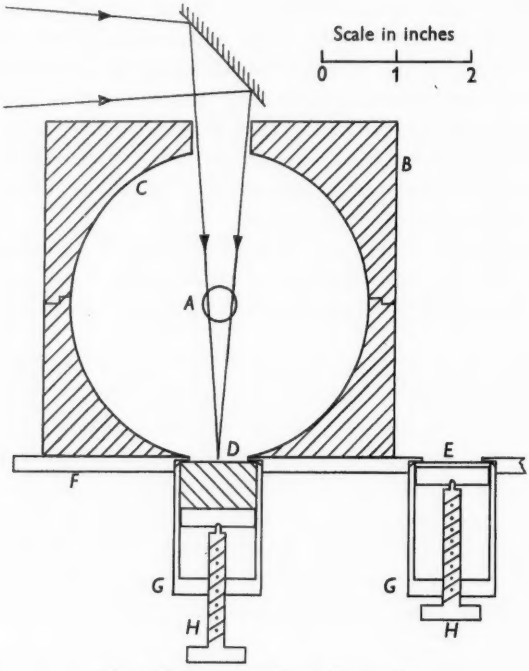

Fig. 1. Integrating sphere reflectometer.

Butter samples were enclosed in the stainless steel cylinders (*G*), fitted with screw plungers (*H*). The edges of the cylinders were sharpened so that the samples could be taken directly from the butter pats by pressing the cylinder into the sample. Preliminary measurements showed that a sample thickness of about 2·5 cm. was adequate for the examination of butter reflectance spectra. The butter surface was prepared for examination by screwing up the plunger slightly and then drawing a knife blade across the butter surface. Using this method, repeated surfaces prepared from a particular butter sample gave reflectance values which did not differ by more than 1%.

Powders were examined in a similar way, the open top of the cylinder being covered with a flat glass plate whilst the powder was slightly compacted by screwing up the plunger. The glass plate was then slid off to leave a flat surface level with the cylinder top. For measurements on dried milk powders, a minimum powder depth of about 5 mm. was required.

RESULTS

Liquid samples

Since water is a major constituent of liquid dairy products, the near infra-red transmission of liquid water at several different thicknesses is shown in Fig. 2. This data has already been published by other workers, but is reproduced here from results obtained with the same absorption cells as were used for examination of liquid dairy products. In this form, interpretation of the spectra of the dairy products is facilitated.

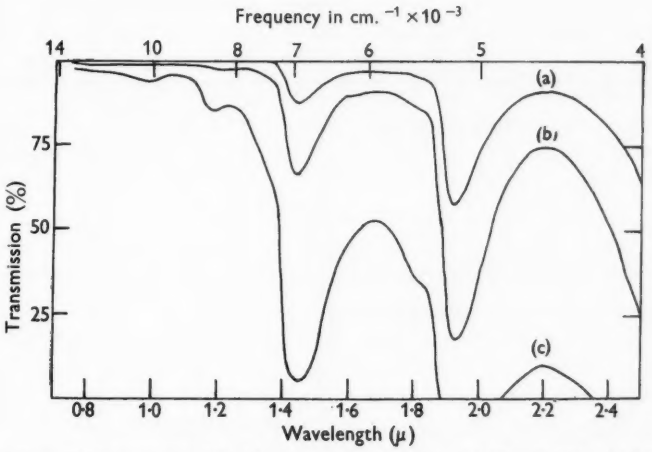

Fig. 2. Transmission spectra of liquid water. Cell thicknesses (*a*) 42μ, (*b*) 135μ, (*c*) 1000μ.

The strong water absorption bands can clearly be seen in the spectra of liquid dairy products (Fig. 3) and butter (Fig. 4). Fig. 3 shows both the reflexion and transmission spectra of liquid dairy products and of butterfat.

No distinct absorption peaks due to components other than water can be distinguished in the transmission spectra of whole milk, homogenized milk, separated milk, or cream, and these spectra can be interpreted in terms of the water absorption bands, together with the effects of the scattered radiation. Loss of radiation due to scattering increased in the order: separated milk, homogenized milk, whole milk and cream. As expected, the scatter losses were considerably less at the longer wavelengths, and at 2·5μ homogenized milk appeared almost as transparent as separated milk.

The reflectance falls off rapidly at higher wavelengths owing to particle size and water absorption effects. Nevertheless, some of the advantages of the reflexion method can be seen by a comparison of the reflexion and transmission spectra of the cream sample. Absorption bands can clearly be seen in the reflexion spectrum, but in the transmission spectrum only the 1·93μ water band can be distinguished and this band does not show clearly above the general background of scattered radiation. Butter samples gave well-defined reflexion spectra (Fig. 4), but it was not found possible to obtain a transmission spectrum of butter in the near infra-red region, owing to the intense scattering; and also to the 'drying-out' of the sample due to strong absorption and consequent heating.

As has already been noted (10), the shorter wavelength absorption bands observed in the reflexion spectra appear to be relatively more intense than the corresponding bands obtained by transmission measurements. This is again demonstrated in the spectra of

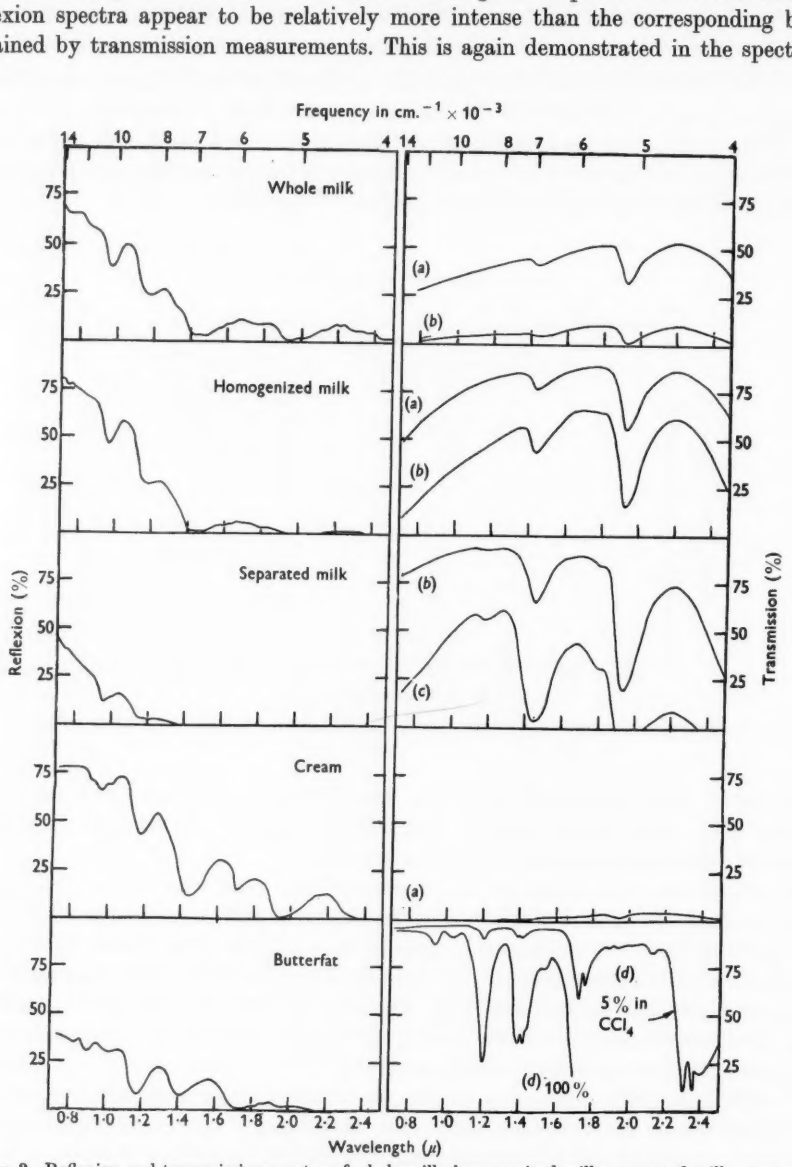

Fig. 3. Reflexion and transmission spectra of whole milk, homogenized milk, separated milk, cream (35% fat), butter fat. Cell thicknesses (*a*) 42μ, (*b*) 135μ, (*c*) 1000μ, (*d*) 1 cm.

Fig. 3, particularly in the case of the bands due to water. The main water bands shown in Fig. 2 can be identified in the milk spectra and in the cream reflexion spectrum. Some of the methylene-group bands can also be identified, although they usually occur as shoulders on the side of the strong water bands.

The butterfat reflexion spectrum (Fig. 3) was obtained at room temperature from a solidified fat sample. Transmission spectra of the same sample were also measured, and these are shown to be very similar to those obtained by Holman and Edmondson for tripalmitin and other related compounds (14). The reflexion spectrum of a finely divided tripalmitin sample has already been published (10). Butterfat is a relatively poor reflector and cooling to 10° C. did not cause much improvement in the general level of reflectance. Owing to the larger slit-widths required when using the integrating sphere, the resolution is not as good as that obtained by the transmission method. It is still possible, however, to observe most of the methylene group absorption bands in the butterfat reflexion spectrum and these correspond to the positions assigned by Kaye (15).

The values obtained here for the reflectance of whole milk and homogenized milk at 0·7 μ are slightly lower than those reported by Lagoni & Merten (2). This is probably due mainly to meniscus effects at the reflecting surface, since these were avoided in Lagoni & Merten's experiments in the visible region by covering the sample surface with a glass plate. Differences in sample composition will also affect the reflectance, and as yet these effects have not been studied in detail.

Dried milk products and butter

These materials scatter considerably more radiation than the liquid samples and reflexion spectra gave much better results in this region of the spectrum. With some difficulty it was possible to obtain transmission spectra of lactose, casein and dried separated milk using the potassium bromide pressed disk technique; but in order to distinguish the weak absorption bands, it was necessary to use relatively large concentrations of sample in the disks. Even after prolonged grinding to ensure small particle size, these disks gave rise to much scattered radiation. By this method it was possible to distinguish the main absorption bands against the general scattering background, and these bands showed at the same wavelengths as observed in the reflexion spectra of the same samples.

In all the spectra shown in Fig. 4, the characteristic absorption bands of the components can be identified against the general falling-off in reflectance at the longer wavelengths due to particle size effects. Table 1 shows the assignments for the main bands in the butter reflectance spectrum and is based upon the data shown in Fig. 3, together with information given in Kaye's structural correlation table (15).

Table 1. *Band assignments for butter*

Wavelength (μ)	Structural group	Wavelength (μ)	Structural group
0·76	H_2O	1·73	$>CH_2$
0·93	$>CH_2$	1·82	H_2O
0·97	H_2O	1·92–2·0	H_2O
1·03	$>CH_2$	2·12	$>CH_2$
1·2	$>CH_2$, H_2O	2·3–2·5	$>CH_2$
1·45	H_2O, $>CH_2$		

The assignments for the dried milk products are shown in Table 2.

Bands assigned to the methylene group in the above table are due mainly to the fat and, to a lesser extent, the casein and lactose components. In these spectra it is not possible to differentiate C—H bonds in the groups CH_3—, $CH_2<$ and $CH<$. Particularly in the case

of the fat component, the relative abundance of methylene groups makes it unlikely that the bands due to C—H bonds in the other groups will be clearly resolved. Strong bands at 1·45–1·6μ, 1·93μ and 2·05–2·15μ assigned to the —OH groups can be seen in the reflectance spectra of other carbohydrates (10). The sharp band at 1·93μ in the lactose spectrum

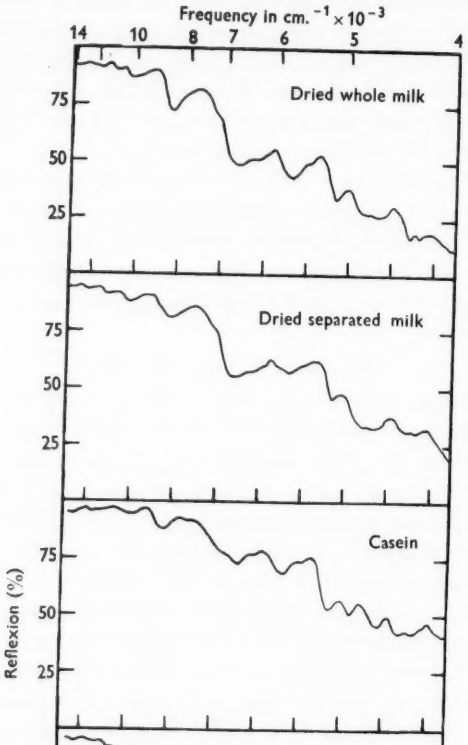

Fig. 4. Reflexion spectra of dried milk products and butter.

is probably due to the water of hydration in the molecule, since this sharp band has also been observed in the reflectance spectrum of hydrated calcium sulphate (10), but is absent in the reflexion spectra of glucose and sucrose (unpublished data).

Table 2. *Band assignments of dried milk products*

Wavelength (μ)	Structural group	Constituents largely responsible for absorption
0·76	—OH, H_2O	Lactose, casein
0·93	$>CH_2$	Fat, casein
~1·0	—OH, H_2O	Lactose, casein
1·18	H_2O, $>CH_2$	Lactose, fat, casein
1·45–1·6	—OH	Lactose, casein
1·73	$>CH_2$	Fat, casein
1·82	H_2O	Casein, lactose
1·93	H_2O, —OH	Casein, lactose
2·05–2·15	—OH	Lactose, casein
2·28	$>CH_2$	Fat, casein
2·32	$>CH_2$	Fat, casein

THEORETICAL INTERPRETATIONS

The theory of light-scattering is complex when applied in detail to the scattering by particles of the same order of size as the wavelengths. Extension of the theory to cover the multiple scattering involved in diffuse reflexion becomes even more difficult, especially when absorption effects must be considered (16). Nevertheless, a qualitative non-mathematical treatment gives some indication of the results to be expected.

As has been shown by Kortüm & Schreyer (17), reduction of the particle size increases the number of scattering centres and leads to an increase in diffuse reflectance. Since the mean path-length within the particles is reduced, a fall in absorption is also observed. This can be demonstrated by the white colour produced from the fine pulverization of blue copper sulphate crystals. As the particle size is still further reduced, a stage will be reached where the particle size becomes so small compared with the wavelength that no scattering occurs.

This is illustrated by the need to reduce the particle sizes of powders when using the potassium bromide pressed disk technique for preparing clear samples for infra-red analysis. Thus it can be seen that as the particle size is reduced, the diffuse reflectance reaches a maximum value and then falls off again. The importance of particle size in a study of the covering power of paints has already been pointed out by Stutz & Pfund (18) and more recently by Sanderson (6).

Pitts, quoted by Burton (4), has shown that, in general, the diffuse reflexion will be considerably less wavelength-dependent than the scattered radiation. A fall in reflectance at higher wavelengths is therefore to be expected, in addition to the effects of absorption which increase rapidly at the higher wavelengths.

It was hoped that this investigation would lead to useful methods for the examination of the globule sizes in milk and butter, and also to information about particle sizes in dried products. Except in the case of homogenized milk, it would seem that the near infra-red reflexion spectrum is not sufficiently sensitive to these factors, and is complicated by the intense absorption effects. Nevertheless, a few practical observations have been made and are reported below.

No mention has been made of the effects of 'anomalous' absorption maxima of the type observed by Stratton & Houghton (19) at $0{\cdot}42\mu$ for a fog composed of particles $2{\cdot}5 \pm 0{\cdot}5\mu$ diameter. It is considered unlikely that these effects will show strongly in the near infra-red region, and it has been possible to explain all the absorption effects in terms of known absorption bands of the components.

PRACTICAL APPLICATIONS

Homogenized milk

During the process of homogenization the larger fat globules are broken up until all the globule diameters are of the order of 1μ. In untreated whole milk the relatively small number of larger globules are responsible for the scattering at longer wavelengths, and the breaking up of these larger globules causes a considerable reduction in scatter, particularly at the $2{\cdot}5\mu$ end of the spectrum. At the shorter visible wavelengths, where the particle sizes are larger than the wavelengths, homogenization leads to an increase in the number of scattering centres with a corresponding increase in reflectance. These two considerations account for the more rapid fall-off in reflectance after homogenization and at $2{\cdot}5\mu$, Fig. 2 shows that homogenized milk is almost as transparent as water, although untreated milk still scatters much of the radiation. Lagoni & Merten (2) have discussed the effects of scatter and reflexion in the visible region, particularly in relation to the degree of homogenization.

Dried milk

As has already been pointed out, the reflexion spectrum of lactose monohydrate shows a sharp band at $1{\cdot}93\mu$, attributed to the molecule of water of hydration. Casein also absorbs at this wavelength, and as expected dried milk samples show absorption at this wavelength. A sample of commercial dried separated milk showed a reflectance of 46% at this wavelength. On standing for several days in a moist atmosphere, the reflectance fell to 35% and the absorption band stood out more strongly in the spectrum. Drying the same sample over phosphorus pentoxide in a vacuum caused only a slight rise of 2% in reflectance. A sample of 'soluble' dried separated milk appeared to give the same reflexion spectrum as a normal sample. No differences were apparent between samples of whole milk powder dried by either the spray or the roller process. A comparison of all the samples so far examined showed no particle size effects of the same order as that already reported for casein samples (10).

Butter

It was originally hoped that the reflexion method would be of value in a study of the structure of butter, since the proportion of solid to liquid fat would be expected to influence the diffuse reflectance. Tests carried out on commercial blended butter samples ($\sim 15{\cdot}5\%$ water content) at 10°, 15° and 20° C. and on dairy butter ($\sim 12{\cdot}5\%$ water content) at 10° and 15° C., showed a general fall in reflectance as the temperature was raised. It is to be expected that a rise in temperature will lead to a fall in reflectance, since at higher temperatures the proportion of fat in the liquid phase is increased and the solid fat will scatter much more radiation than the liquid fat. Changes in reflectance observed in the $0{\cdot}8\mu$ region were only about 6% for a 10° C. temperature rise.

The reflectance at the $1{\cdot}93\mu$ water band was too low to attempt a correlation with water content, but it was observed that at the flatter portion of the curve near $0{\cdot}8\mu$, the reflec-

tance of unsalted butter samples was noticeably greater than that of salted samples, as shown in Table 3.

Table 3. *Effects of salt on butter reflectance at* $0{\cdot}8\mu$

Sample	Average water content	Reflectance at $0{\cdot}8\mu$ (%)	
		Salted butter	Unsalted butter
Commercial (blended)	15·5	86	90
Dairy	12·5	74	81

It is difficult to account for these differences other than by the suggestion that the introduction of salt affects the globule structure in some way.

As expected, the reflexion spectra of blended butter and dairy butter showed similar effects to those observed for homogenized and untreated whole milk. In general, blended butter samples reflect about 10% more radiation than dairy butter at $0{\cdot}8\mu$, but it is possible that these differences could be due solely to the different water and salt contents. Margarine samples gave a reflexion spectrum very similar to that observed for blended butter samples.

SUMMARY

A method for the study of the diffuse reflexion spectra of dairy products in the near infrared region has been described. It has been shown that in this region of the spectrum, the reflexion method gives better results than the transmission method for butter and dried milk products. The effects of homogenization on milk spectra have been studied, and also the effects of temperature and mechanical working on the spectrum of butter have been considered. Assignments have been made for the bands observed in the dried milk spectra, and the effects of moisture and other factors on the spectra of dried materials have been briefly studied.

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REVIEWS OF THE PROGRESS OF DAIRY SCIENCE

SECTION A. PHYSIOLOGY OF DAIRY CATTLE

HORMONES IN REPRODUCTION AND LACTATION

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INTRODUCTION

The object of this article is to review studies on the role of hormones in the physiology of reproduction and lactation in dairy cattle, published since 1953 when Smith last surveyed this field in the *Journal*; work on species other than ruminants will be considered only when this seems helpful to the understanding of physiological mechanisms in the ruminant. Within the last few years several more general reviews dealing with the role of hormones in reproduction have appeared, the more important of these being the papers presented at a Conference on Female Reproduction in Farm Animals at the Iowa State College (*Iowa State College Journal of Science*, **28**, no. 1, 1953), the *Proceedings of the Centennial Symposium on Reproduction and Infertility at the Michigan State University* (1955), a number of the *British Medical Bulletin* (**11**, no. 2, 1955) devoted entirely to reviews on 'Hormones and Reproduction', and the *Proceedings of the 3rd International Congress on Animal Reproduction* (1956). Another part of the 3rd edition of *Marshall's Physiology of Reproduction* (vol. **1**, pt. 1, 1956) has also recently been published. In the field of lactational physiology, the most important review of recent years has been the book by Folley (1956) on the *Physiology and Biochemistry of Lactation*. To these and other specialist reviews frequent references will be made.

THE HORMONES IN BODY FLUIDS OF RUMINANTS

Although there has been much speculation on endocrine functions and dysfunctions in reproduction in the cow, factual information on the nature and amounts of hormone substances present in the ruminant body has until recently been almost non-existent. This paucity of data has been in marked contrast to the wealth of information about the hormones and their metabolites in women and in mares. In these species relatively large quantities of hormones or hormone derivatives are excreted in the urine, particularly at certain stages of pregnancy, and this has greatly facilitated their identification and assay. Ruminants, however, are much less extravagant in

their excretion of hormones, or of hormone derivatives in a recognizable form, and it is only of recent years that serious attempts have been made to overcome the difficulties of hormone identification and assay in these species. In the past the difficulties have not always been fully appreciated, nor the danger realized, of applying to the ruminant methods of assay developed for other species. In discussing the study of urinary steroids in the ruminant, Klyne & Wright (1957) have stated two important propositions which undoubtedly have a general application to hormone studies in the cow: first, a thorough qualitative investigation of the urinary steroids should be made before their estimation is undertaken; and secondly, care must be taken in applying to animal urines, which may contain unknown interfering constituents, methods designed for human urine.

Oestrogens

The earliest hormone assays in the cow were concerned with the urinary excretion of oestrogen in pregnancy (for references see Cowie, 1948). Such bioassays indicate the quantity of the actively oestrogenic substances but tell us nothing of the chemical nature of the oestrogens present nor do they indicate the presence of biologically inactive oestrogen derivatives. Recently, Smith, Dickson & Erb (1956) have assayed the oestrogen in bovine urine by both chemical (fluorimetric) and biological methods. The oestrone equivalents per litre obtained by the chemical procedure were some 3·7 times as high as those obtained by bioassay.

While assays by the present chemical and biological methods may give useful information on major variations in oestrogen excretion during the various reproductive phases, significant advances await the identification of the compounds measured by these assay procedures. It is pleasing to note that some progress towards this goal is now being made.

In a comprehensive study of the steroids and other lipids in the urine of the pregnant goat, Klyne & Wright (1957) have isolated and identified oestrone and oestradiol-17α in concentrations of 0·3 and 0·1 mg/l. respectively. No evidence for the presence of oestradiol-17β was obtained. Preliminary reports indicate that oestrone and oestradiol-17α are present also in the urine of the pregnant cow in somewhat similar concentrations, again no detectable amount of the 17β epimer was present (Klyne & Wright, 1956). In view of the probable absence of oestradiol-17β from the urine it is of interest to recall that strong evidence for the presence of oestradiol-17β in cow faeces was reported by Levin in 1945. Oestrone has also been isolated from cow urine by Pope, McNaughton & Jones (1957). The presence of oestradiol-17α may well explain the discrepancy between the chemical and bioassay methods reported by Smith *et al.* (1956) since oestradiol-17α is to all intents and purposes oestrogenically inactive. It is, moreover, possible that it might interfere with the assay of the active oestrogens since it has been shown that oestrogens of low biological activity may modulate the action of more active oestrogens (Hisaw, Velardo & Goolsby, 1954). Large quantities (5–15 mg./l.) of the heterocyclic phenol, equol (*iso*-flavan-7:4-diol), are present in pregnant cow and goat urine (Klyne & Wright, 1956, 1957); this compound is almost certainly of dietary origin, it has no biological activity but may interfere in chemical assays of oestrogens. The development of suitable chemical assays for oestrone and oestradiol-17α in ruminant urine is desirable so that the excretion of these steroids under varying physiological conditions and in cases of reproductive failure may be studied.

A few studies have been reported on the oestrogens present in the blood of cows. Bioassays of extracts of blood from two pregnant cows have revealed the presence of oestrogenic activity equivalent to 0·36 and 0·38 μg oestradiol-17β/100 ml.; two-thirds of the total oestrogen was closely associated with the protein fraction of the serum, the remaining third existed mainly in a hydrophilic non-protein conjugated form (Szego & Roberts, 1946). Dr G. S. Pope (personal communication) has confirmed the presence of oestrogen in cow blood equivalent to about 0·1 μg. oestradiol-17β/100 ml. Both ketonic and non-ketonic oestrogens are said to be present in bovine blood (Asdell, 1952).

Pope & Roy (1953) reported that in bovine colostrum oestrogens, partly ketonic, are present mainly as conjugates in the aqueous phase of the colostrum equivalent to about 5 μg. oestradiol-17β/l. Later work has indicated that some, though not the major part, of the oestrogenic activity

is due to a substance which from its chromatographic properties and microchemical reaction is believed to be oestrone (Dr G. S. Pope, personal communication). Pope & Roy (1953) could detect no oestrogens in normal milk, but Münch (1954), using an extraction procedure, detected oestrogenic activity in milk from pregnant cows.

Significant quantities of oestrogen do not appear to be present in bovine cervical mucus (Dr G. S. Pope, personal communication).

Progestogens

Progesterone has not been detected in urine, but in women the urinary pregnanediol is generally regarded as providing an index of the progesterone levels in the circulation. An early claim by Marker (1938) that considerable quantities of pregnanediol were present in the urine of pregnant cows has never been substantiated (for early references see Cowie, 1948; also Hill, Petersen & Cohen, 1954). In a recent comprehensive study of the steroids of bovine urine, Klyne & Wright (1956) have been unable to find *any* isomer of pregnanediol and they suggest that the pregnanediol found by Marker may have been introduced by faecal contamination since pregnanediols are present in bovine bile (see below). The common pregnanediol of human pregnancy urine (i.e. 5β-pregnane-3α:20α-diol) has been isolated in small quantities from the urine of the pregnant goat, the excretion rate being about 5 mg./day; no other isomer could be demonstrated (Klyne & Wright, 1957). 5β-Pregnane-3α:20α-diol has also been isolated from the urine of pregnant ewes (Dr H. Robertson and Dr W. F. Coulson, private communication). After the injection of large quantities of progesterone daily (100 mg.) into the goat no significant quantities of pregnanediol or other recognizable progesterone metabolites could be isolated from the urine (Boscott, 1950).

It may be concluded therefore that if pregnanediol be a metabolite of progesterone in the ruminant, it is not excreted to any appreciable extent in the urine.

The assay of progesterone in the blood and other body fluids is difficult. The progestogenic activity of the blood can be measured by a bioassay procedure developed by Hooker & Forbes in 1947, or progesterone may be estimated by chromatographic procedures.

Neher & Zarrow (1950, 1954), using the Hooker & Forbes assay, detected the equivalent of 0·3–2 μg./ml. progesterone during oestrus, and 6 μg. in the luteal phase, in the peripheral blood of ewes, and 6–12 μg./ml. in the blood of ewes in late pregnancy and at parturition. These relatively large quantities have not been found when chromatographic methods have been used. Edgar (1953, 1954) was unable to detect progesterone in the peripheral blood of pregnant cows or ewes, although he did find it in the blood draining from the active ovary in both the pregnant and non-pregnant ewe. These observations have been extended by Raeside & Turner (1955), who detected progesterone in the blood leaving the ovary of a pregnant goat but not in the blood from the uterus; no progesterone was found in the peripheral blood of the goat, sheep or bovine. Recently, however, Short (1957) by a chromatographic method has detected progesterone in the peripheral blood of pregnant and non-pregnant cows and sheep. To explain the discrepancy between bioassay and chemical procedures, Edgar has suggested that the bioassay may be measuring other progestogenic substances as well as progesterone. This view has received support from subsequent work by Zarrow & Neher (1955), who consider that the normal progestational hormone is not progesterone *per se* but possibly an allied steroid or progesterone combined with another substance.

Experiments by Neher & Zarrow (1954) suggested that the placenta was the major source of progestogen in the ewe during the last two-thirds of pregnancy, but progesterone has not been detected in late-term placentae of the cow or ewe (Short, 1956). The progestogen content of the plasma in the umbilical artery of the goat foetus is appreciably higher than in the plasma of the umbilical vein, which suggests a production of progestogen by the foetus and its removal from the blood by the placenta (Forbes, 1955).

Further investigations are necessary to resolve the fundamental question of the identity of the progestogenic substances in the blood and placentae. In this connexion it may be of some

interest that certain 20-hydroxy derivatives of progesterone have been isolated from human and equine placentae (Short, 1957), and one of these (20α-hydroxypregn-4-en-3-one) possessing significant biological activity has now been isolated from the blood of pregnant sheep (Dr R. V. Short, private communication).

A clue has recently been provided as to how progesterone or the progestogenic hormone is excreted in the ruminant. The presence of androgenic substances in cow faeces was reported some years ago (132, 227, 228, 281), and since these androgens are present in the greatest concentration in the faeces of sexually mature or pregnant cows, Miller & Turner (1955) postulated that these androgens might be related to progesterone metabolism. They injected seven bovines with 1 g. progesterone per day, and in six cases there was a significant increase in the output of androgens in the faeces, in the seventh case—a pregnant cow—the increase was not significant. In further studies the identities of the faecal androgens were established as 5α-androstane-3:17-dione, androst-4-ene-3:17-dione and androsta-1:4-diene-3:17-dione; 5β-androstane-3:17-dione (etiocholane-3:17-dione) was also present (Miller, Turner, Fukushima & Salamon, 1956). The site of the metabolic transformation of progesterone in the ruminant body is at present not clear. Pearlman & Cerceo (1948) found 3α-hydroxy-5β-pregnan-20-one, 5β-pregnane-3α:20β-diol and 5β-androstane-3α:17α-diol (called etiocholanediol-3α:17β by these workers) in cow bile but they could detect no progesterone. It may be that progesterone is converted into these steroids in the liver or elsewhere in the body and that these steroids in turn undergo a microbiological transformation in the gut to the above-mentioned androgens.

Ketosteroids

Estimation of urinary 17-ketosteroids in the human subject gives information on the end-products of the metabolism of the C_{19}-steroids which have arisen either from the gonads or the adrenal cortex. In considering ketosteroid estimations in the ruminant it is necessary to emphasize a most important observation made by Holtz (1954, 1956) and Klyne & Wright (1957). If the usual Medical Research Council (1951) method for 17-ketosteroid determinations based on the Zimmermann colour reaction be applied to ruminant urine *wholly false results will be obtained*. By a fortunate chance the Zimmermann chromogens in human urine are very largely 17-ketosteroids; in ruminant urine, however, there are present large quantities of C_{13}-compounds related to ionone, presumably of dietary origin, some of which give a strong Zimmermann colour reaction. Because of these interfering substances, the quantities of ketosteroids reported in some early studies are quite unreliable. In pregnant goat urine, the Zimmermann procedure gave apparent ketosteroid values of 7–10 mg./l., although the true value was about 1 mg./l. (Klyne & Wright, 1957). In cow urine, Holtz (1956) has reported a true value of approximately 0·3 mg./l. The individual ketosteroids present in ruminant urine have not been fully characterized, but Klyne & Wright (1957) have isolated *epi*-androsterone from pregnant goat urine and have also obtained evidence of the presence of androsterone, aetiocholanolone and its 11-keto derivative.

An unidentified ketosteroid has been isolated from the adrenal venous blood of the cow (Gassner, Nelson, Reich, Rapala & Samuels, 1951) and 11β-hydroxy-androst-4-ene-3:17-dione has been identified in the adrenal venous blood of the ewe (Bush & Ferguson, 1953).

Corticosteroids

One of the first attempts to measure blood corticoids in the ruminant was that of Reich, Nelson & Gassner (1951), who devised a special surgical method for the cannulation of the adrenal sinus in the cow so that the venous effluent could be collected. In this effluent they identified cortisol. Two years later Bush & Ferguson (1953) collected venous blood from the adrenal of normal and hypophysectomized anaesthetized sheep and reported that the predominant corticosteroid was cortisol; corticosterone was also present. More recently attempts have been made to measure adrenal steroids in the peripheral blood of ruminants. Forsius & Haikonen (1955), using a modification of the Nelson-Samuels procedure, found 17-hydroxycorticoids in the blood of sheep

equivalent to 0–4·5 μg cortisol/100 ml. plasma. Robertson & Mixner (1956), using a modification of the Porter-Silber reaction, made extensive studies in cows; 17-hydroxycorticoids equivalent to a mean value of ~10 μg. cortisol/100 ml. plasma were found in the blood of twenty-three non-lactating pregnant cows, a mean of ~5 μg. cortisol/100 ml. plasma for twenty lactating non-pregnant cows. Robertson & Mixner observed that certain chromogenic substances are present in cow blood which interfere with the assay if the Nelson-Samuels procedure were used (a procedure also based on the Porter-Silber reaction). Further studies of the levels of 17-hydroxycorticoids in bovine blood during the various phases of the reproductive cycle are awaited with interest.

It is now well-established that changes in the levels of 17-hydroxycorticoids in the blood affect the numbers of lymphocytes and eosinophils in circulation. While lymphopenic and eosinopenic responses are not specific for increases in blood 17-hydroxycorticoids, these responses always do occur after adrenal stimulation by adrenocorticotrophin (ACTH) or the administration of 17-hydroxycorticoids. In human medicine the value of the eosinophil count has been well-established (see Thorn, Jenkins & Laidlaw, 1953), and provided the limitations are recognized eosinophil and lymphocyte counts are useful indicators in studying changes in levels of 17-hydroxycorticoids in the circulation in ruminants. There are lymphopenic and eosinopenic responses at parturition in the cow (184, 223, 313) and in the goat (Cowie, unpublished). The effect of ACTH on the eosinophil count in the cow has been studied by Flux, Folley & Rowland (1954) and Andersson (1954). Concurrent increases in plasma 17-hydroxycorticosteroids and decreases in the eosinophil count of cows after stress have been reported by Saroff & Turner (1956).

There have been several attempts to measure the urinary excretion of corticosteroids in ruminants, but some of the results are of doubtful significance in view of possible inadequacies in the assay procedures when applied to ruminant urine. Using the Sprechler modification of the Heard & Sobell technique, Holcombe (1953, 1956) studied the excretion of 'reducing corticoids' in the cow during pregnancy and observed a high excretion in the last month of pregnancy equivalent to 8·2 μg. deoxycorticosterone acetate per litre. No cortisone and only traces of cortisol were found in the urine of a ewe receiving a 3-day infusion of ACTH (Wynne, 1954). Holm & Firch (1955) concluded that 17-hydroxycorticoids (modified Porter-Silber assay procedure) were not normally excreted by the cow, even in pregnancy, but they were found in the urine after surgical trauma. Since the Porter-Silber reaction is considered to be relatively specific for 17:21-dihydroxy-20-ketosteroids, it would appear that steroids of this group do not constitute a significant portion of the total 'reducing corticoids' in ruminant urine, whereas in human urine they may constitute about 25% of the 'reducing corticoids' (Sprechler, 1955). Further studies on the identity of the corticoids in ruminant urine are necessary.

Some preliminary studies have been made on the metabolism of corticosteroids in ruminants. After the intravenous injection of cortisol (0·5–1·0 mg./lb. body weight) into sheep and cattle there is a rapid clearance of 17-hydroxycorticosteroids from the blood, the values returning to normal usually within 1 hr., only 0·18–0·77% of the injected dose being recovered in the urine (Holm & Firch, 1956). No corticosteroids have been recovered from the faeces of a normal ewe (Wynne, 1954), nor are significant quantities of 17-hydroxycorticosteroids present in the bile of the ewe following intravenous injection of cortisol (Holm & Firch, 1956), although large quantities (equivalent to 600 μg. cortisol/l.) were found in the bile of a ewe *in extremis* (Wynne, 1954).

Relaxin

Of late there has been renewed interest in this ovarian hormone. Contrary to an early report (Hisaw, 1929) relaxin has now been detected in the blood of the pregnant cow. Wada & Yuhara (1955) studied nine cows throughout pregnancy and found that the relaxin concentration in the blood rose from 1 guinea-pig unit (g.p.u.) per ml. serum at the end of the first month to a level of 4 g.p.u. per ml. at the 6th month: the concentration remained at this level until a few hours after parturition when it dropped rapidly to 1 g.p.u. by the 24th hour, and disappeared by the 6th day *post partum*.

Oxytocin

A procedure has recently been developed for the assay of oxytocin in human blood (Bisset & Walker, 1954; Bisset & Lee, 1956). The application of this assay to the study of oxytocin levels during the milking process in the cow would be most interesting.

The presence of an enzyme in the blood of pregnant women which inactivates oxytocin was first described by Fekete in 1930, and his observations have since been confirmed by other workers (for references see Cowie, 1948). The enzyme, however, was not detected in the blood of the cow (Werle, Hevelke & Buthmann, 1941), although an oxytocinase has recently been found in bovine mammary tissue (Werle & Maier, 1954).

Gonadotrophins

Significant quantities of gonadotrophins have not been detected in the blood or urine of ruminants (for references see Cowie, 1948). More recently, studies of blood samples of sheep during various phases of the oestrous cycle have failed to reveal gonadotrophic activity even though large volumes (500 ml.) of blood were tested (Bassett, Sewell & White, 1955).

Recent improvements in the methods of isolating gonadrotrophin for human urine and of detoxicating such preparations have made it possible to administer to a rat for assay purposes the equivalent of 1 l. of urine (Loraine & Brown, 1956). The re-investigation of bovine urine using such procedures, or suitable modifications, for the presence of small quantities of gonadotrophin might yield important results.

The claim by Bhaduri and his colleagues (Bhaduri & Bardhan, 1949) that the faeces of pregnant cows contains a gonadotrophic substance, as evidenced by the ability of aqueous extract of faeces to release sperm in male toads, has aroused considerable interest. Subsequent investigations have confirmed that extract of bovine faeces may cause spermiation in toads, but the effect is not confined to extracts of faeces of pregnant cows, and it is generally agreed that the reaction is not sufficiently reliable to form the basis of a pregnancy test (11, 60, 150, 271). The possibility that the gonadotrophin-like substance might be of dietary origin was put forward by Cowie (1951), and it was later shown by Harris (1952, 1953) that extracts of cattle feedingstuffs did indeed release sperm when injected into male toads. The reaction has also been attributed to the presence of oxalates in the faeces (Tabarelli Neto, Salles Gomes & Fernandes Filho, 1954). A more thorough investigation of the nature of the active material has recently been made by Banik & Reineke (1955), who found that the active substance was not precipitated from aqueous solution by acetone and that it was comparatively heat-stable even to boiling for 1 hr. Purified extracts, while active in toads, had no gonadotrophic effects when injected into oestrous rabbits. Whatever the active substance may be, Banik & Reineke have shown that it differs from the known gonadotrophins and from the androgens present in bovine faeces. Further particulars of Bhaduri's experiments will be found in the following papers (15, 24, 25, 26, 28, 29, 30, 31).

Hormones in semen

Both androgens and oestrogens have been detected in bull semen, but their physiological significance is not yet evident (Puntriano, 1953; Schaffenburg & McCullagh, 1954). The effects of various steroids and related substances on the *in vitro* metabolism of bull sperm have been studied by Gassner & Hopwood (1955). Further references and discussion of this topic will be found in the comprehensive review of the biochemistry of semen by Mann (1954).

HORMONE CONTENT OF THE PITUITARY

The significance of the hormone content of endocrine glands has been the subject of much debate. In an excellent review on the gonadotrophic activity of the pituitary gland, Nalbandov (1953) concludes that assays of the gonadotrophin content do 'indeed measure the rate at which

the gonadotrophic complex is being produced and secreted into the blood stream at time of autopsy of the animal'. In considering the same question Cowie & Folley (1955), however, found the evidence not entirely convincing and concluded that 'the concept that the gonadotrophic potency of the pituitary reflects the rate of liberation into the blood stream of the hormones must be regarded with some reserve. It may do so only under certain conditions, and experiments on blood levels of gonadotrophins are necessary to answer this question.'

Kammlade Jr., Welch, Nalbandov & Norton (1952) observed that the total gonadotrophic potency of the pituitary of the sheep was uniformly higher during the non-breeding season than during the breeding season. There was a distinct and significant drop in gonadotrophic activity at the time of the first oestrus followed by a gradual increase in potency to the 16th day of the cycle. Cole & Santolucito (1956) have made a preliminary report on assays of follicle-stimulating hormone (FSH), luteinizing hormone (LH) and prolactin in the sheep pituitary during the oestrous cycle. A precipitous drop in FSH occurred between the 4th and 25th hour after the beginning of oestrus, during the same period LH and prolactin increased in amount. The significance of these findings must remain obscure until the pattern of hormone levels in the blood is known. Of importance with reference to assays of LH in pituitary tissue is the observation of Segaloff, Flores & Steelman (1955) that the presence of prolactin may interfere with the response in the ventral-prostate weight test for LH in hypophysectomized rats.

A study of the gonadotrophin content of the pituitary of the hermaphrodite goat has been made by Naito (1953).

No significant changes in the thyrotrophic potency of the pituitary have been observed in sheep during the oestrous cycle or during anoestrus (Kammlade Jr. *et al.* 1952). Assays of growth hormone (GH) and thyrotrophic hormone (TSH) in the anterior pituitary of heifers raised on three levels of nutrition and autopsied at six different age periods have been made by Armstrong & Hansel (1956). The GH per g. of the anterior pituitary or per 100 lb. body weight, and the total TSH were greater in young heifers and gradually decreased with age; no significant effects due to the plane of nutrition were detected. Since the rates of body growth during 16 weeks prior to slaughter were positively correlated with GH per g. anterior pituitary and per 100 lb. body weight and the thyrotrophic potencies were also positively correlated with thyroid weights, it is considered that the quantities of GH and TSH present in the pituitary reflect the actual rate of secretion of these hormones. Studies on the GH and TSH content of the cow pituitary during lactation do not yet appear to have been made.

Cellular origin of the pituitary hormones

The experimental evidence on the cellular origin of the gonadotrophic hormones and prolactin in various species has recently been reviewed by Cowie & Folley (1955). A more detailed discussion of adrenohypophysial functional cytology with special reference to the bovine pituitary will be found in the review by Jubb & McEntee (1955) who have made also a comprehensive histological and histochemical study of the bovine pituitary during various phases of the reproductive cycle. Using the periodic acid Schiff technique (PAS) cells were classified into β and δ types on the basis of positional, morphological and functional differences. The δ cells are believed to be concerned with gonadotrophin secretion—LH in particular. These cells showed cyclic changes during the oestrus cycle, degranulation occurring in the first hours of oestrus. In cows which came into oestrus but failed to ovulate these changes were not observed. The β cells are believed to secrete TSH. Jubb & McEntee have so far been unable to subdivide the acidophils or distinguish between those producing GH and those producing prolactin. The acidophils discharged their granules at about the 3rd day after oestrus and reached a peak of degranulation at the 10th day. Hypertrophy and the signs of increased activity were observed in the acidophils from the 4th to the 8th months of pregnancy. The investigation of Jubb & McEntee appears to be a very significant advance in the study of the functional cytology of the bovine pituitary.

HORMONAL CONTROL OF THE OESTROUS CYCLE AND FERTILIZATION

The physiology of the gonadal cycles in domestic animals has been discussed at length by Gassner (1952) and Amoroso (1955), and the problems of the control of pituitary gonadotrophic function have recently been considered by Cowie & Folley (1955).

It has been customary to consider the rhythm of events in the oestrous cycle as being due in part to a hormonal interplay between the ovaries and anterior pituitary. The increasing level of oestrogen in the blood associated with the developing follicle was said to inhibit the release of FSH and to facilitate the secretion of the LH responsible for ovulation. In turn, the secretion of LH was believed to be inhibited by increasing progesterone levels in the blood as the corpus luteum developed. It is becoming evident, however, that in the ruminant the pattern of hormonal interplay differs considerably from the above which was derived mainly from studies in laboratory animals. There is, moreover, evidence that the central nervous system exerts a considerable control over pituitary gonadotrophic function via the hypothalamus. The nature of the controlling links between hypothalamus and anterior pituitary are still matters of controversy, and the reader is referred to the excellent reviews by Zuckerman (1954) and Harris (1955) for further details.

Neurohormonal factors

That a neural factor might participate in the control of ovulation in the bovine was suggested by the observation that sterile copulation significantly reduced the interval between the onset of oestrus and ovulation in heifers (Marion, Smith, Wiley & Barrett, 1950). Further evidence was forthcoming when Hansel & Trimberger (1951) showed that ovulation could be inhibited in heifers by injecting atropine at the beginning of oestrus. Since ovulation was not delayed if LH was administered with the atropine, it may be assumed that the atropine was not acting directly on the ovary but on some neural factor having a cholinergic component regulating the release of LH from the pituitary. It was further shown that the injection of progesterone in low doses (5–10 mg.) at the onset of oestrus hastened ovulation in heifers by reducing the duration of oestrus and the interval between the end of oestrus and ovulation (Hansel & Trimberger, 1952). Injections of oestradiol, on the other hand, had no effect on the time of ovulation. In subsequent studies, Hough, Bearden & Hansel (1955) showed that progesterone was not acting directly on the anterior pituitary, since the blocking effect of atropine on ovulation could not be overcome by the simultaneous administration of progesterone, but was exerting its action at the hypothalamic level. These findings are not in keeping with the concept that increasing oestrogen levels in the blood stimulate the release of LH while progesterone suppresses LH. Hansel (1953) suggests that a pre-ovulatory rise in progesterone may be an integral part of the ovulatory mechanism in the cow. In harmony with this view is the detection of progesterone in the follicular fluid of the cow just prior to ovulation (Edgar, 1953). In the sheep, moreover, ovulation has been induced during the anoestrous period by the administration of progesterone alone, indicating that progesterone can participate in the release of LH (86,99,285,287).

At other times in the oestrous cycle, progesterone can inhibit both oestrus and ovulation in the bovine. Doses of 50 mg. daily from the 15th day of the cycle inhibit oestrus and ovulation, although some follicular development takes place; lower doses (25 or 12·5 mg.) allow greater follicular development while usually suppressing oestrus and ovulation; doses less than 12·5 mg. are generally ineffective in suppressing ovulation and oestrus (Ulberg, Christian & Casida, 1951). A very low conception rate is obtained if animals so treated are mated at the first oestrus after progesterone administration. The duration of the next oestrous cycle is, however, normal, and if the animals are bred at the second oestrus the conception rate is normal (Trimberger & Hansel, 1955).

Oestrous behaviour

Within the last few years there have been interesting developments in the analysis of the hormonal factors necessary for the exhibition of normal oestrous behaviour. This work has been

done on sheep, but it will undoubtedly provide a basis for future studies in the cow. Robinson (1954*b*) failed to produce normal oestrous behaviour in the ovariectomized ewe by the administration of oestrogens alone, the behaviour tending to be male-like in character. While an oestrus-like condition was induced in spayed ewes injected with large doses of oestradiol benzoate (2 or 5 mg.), repetition of the injections at 7- or 14-day intervals produced a refractory condition and the percentage of ewes showing oestrus-like behaviour declined rapidly. If the ewes were pre-treated with progesterone (25 mg. daily for 3 days), the quantity of oestrogen necessary to induce heat was greatly reduced, 40 μg. oestradiol benzoate being sufficient for a maximal response. Oestrous behaviour, moreover, was normal and no refractory period developed. Robinson concluded that oestrous behaviour in the sheep is under dual hormonal control and that progesterone conditions receptor tissues in the central nervous system to respond to low levels of oestrogen. Subsequent studies (232, 288, 290, 291) demonstrated that for the full expression of the progesterone—oestrogen interaction in the manifestation of oestrus, ewes had to be under the influence of progesterone for about 12 days. It would therefore appear that the cycling ewe is dependent on progesterone arising from the corpus luteum formed at the site of the previous ovulation, and not to progesterone from the developing follicle, for becoming suitably conditioned to show oestrus at the next cycle. That such conditions probably exist in the ewe is borne out by the report of Edgar (1954) that detectable amounts of progesterone appeared in the blood from the active ovary at the 3rd or 4th day of the cycle; the quantity increased till the 9th day, remained high to the 16th day and fell suddenly on the 17th day. From their experiments, Robinson and his colleagues estimate that a ewe showing a 16-day cycle must produce oestrogen equivalent to a single injection of 25 μg. oestradiol benzoate to show normal oestrus and the associated cellular changes in the vagina.

The role of progesterone in eliciting normal oestrus has been further emphasized by experiments on the out-of-season breeding in sheep. Injections of pregnant mares' serum gonadotrophin (PMS) have long been used for this purpose (for references see Cowie & Folley, 1955), but recent work has shown that ovulation is more often preceded by overt oestrus if progesterone is administered prior to PMS (86, 99, 194, 284, 285, 287).

The above-mentioned studies indicate that progesterone plays important roles both in the regulation of ovulation and the manifestation of normal oestrous behaviour in the ruminant.

Cervical secretions

The physical properties of the cervical secretions of the cow have been the subject of considerable research, as the secretions may be important in facilitating sperm travel, and changes in their physical properties may be used in the detection of oestrus and pregnancy. There is evidence that the alterations in physical properties are under hormonal control (for early references see Cowie, 1948). Recent studies on the flow properties of bovine cervical secretions have shown that cyclic variations occur which are correlated with the oestrous cycle, and other changes were noted after conception (Glover & Scott Blair, 1953; Scott Blair & Glover, 1956). A promising test for pregnancy in the cow appears now to be emerging from these studies (Scott Blair & Glover, 1955). Experiments are at present in progress to ascertain in ovariectomized cows the hormonal complexes responsible for these changes in physical characteristics. A mucus of oestrus-type consistency was obtained when 0·8–2·0 mg. hexoestrol was injected subcutaneously for 3 days; 0·5 mg. hexoestrol in combination with progesterone in doses from 0 to 70 mg. per day resulted in increases in consistency with increasing progesterone levels, a pregnancy-type secretion being obtained with 30 mg. progesterone (Mr F. A. Glover, personal communication). Progesterone is also responsible for the thickening of the cervical mucus in sheep (Robinson & Moore, 1956).

Crystallization patterns. Crystallization patterns of cervical mucus of the cow change during the ovarian cycle and pregnancy (47, 130, 156, 157, 268). It has been claimed that provided certain precautions be observed the crystallization patterns may give an indication of pregnancy within a few weeks of conception (Higaki & Awai, 1953*c*; Bone, 1954). The changes in pattern are believed

to be associated with alterations in oestrogen and progesterone levels in the blood, and the type of pattern is correlated with the flow properties of the mucus (Mr F. A. Glover, personal communication).

Histology and histochemistry of the reproductive tract and allied endocrine glands

There have appeared recently several interesting and comprehensive studies on the histology and histochemistry of the reproductive tract and related endocrine glands of the cow (83, 116, 235, 236, 237, 238, 322, 336), of the pituitary, gonads and adrenals of the bull (Rollinson, 1954), of the seminal vesicles of the bull (Cons, 1957), of the thyroid and testis of the buffalo (Bhatnager, Mukherjee & Bhattacharya, 1955) of the oviduct of the ewe (Hadek, 1955), of the pituitary of the hermaphrodite goat (Naito, 1953) and of the reproductive organs of the male goat (Yao & Eaton, 1954). Further information on hormone levels in the blood is necessary before the precise role of hormonal factors in histological and histochemical changes in the reproductive tract can be assessed.

Maintenance of the corpus luteum in pregnancy

The essential role of progesterone during pregnancy in the cow has been established (McDonald, Nichols & McNutt, 1952, 1953), but little is known of the factors which maintain the functioning of the corpus luteum after fertilization or, as the question is posed by Moore & Nalbandov (1955), 'how does the ovary know that the uterus is pregnant?'.

In the rat there is considerable evidence that prolactin maintains the functional activity of the corpus luteum, but there is as yet no unequivocal evidence that it can do so in other species (see review by Cowie & Folley, 1955), although some recent experiments suggest that prolactin may be luteotrophic in the ewe (Moore & Nalbandov, 1955).

If it be accepted that prolactin, possibly in conjunction with other pituitary hormones, is responsible for the maintenance of the corpus luteum, the question as to how these hormones are released from the pituitary still requires to be answered. Experiments by Nalbandov and his colleagues (Moore & Nalbandov, 1953; Nalbandov, Moore & Norton, 1955) appear to suggest a possible answer. They have shown that a foreign body (an 8 mm. plastic bead), when introduced into the lumen of the uterus of a ewe during the early luteal phase of the cycle (3rd day), shortens the duration of the oestrous cycle, whereas if it is introduced in the late luteal phase (8th day) the cycle is considerably lengthened. These effects were abolished—the cycle becoming normal—when the uterus, or that part of it containing the bead, was denervated. Stimuli from the uterus using neural or neurohumoral pathways can thus influence anterior-pituitary function, and such factors may well operate in early pregnancy. The physiological significance of the effects of hysterectomy in the cow and ewe on the corpus luteum as described by Wiltbank & Casida (1956) is not clear.

Experimental control of ovulation

Hormones have been used in animal husbandry in various ways to regulate ovulation in ruminants. The more important of these applications are: (1) the breeding of sheep and goats during the anoestrous season to obtain an extra crop of lambs, or to increase the supply of goat's milk in the winter months; (2) the production of superfoetation in ewes in the normal breeding season to increase the fertility of the flock; (3) the production of superovulation to obtain ova for transplant experiments; (4) the synchronization of oestrus in groups of animals to facilitate artificial insemination or ova transplantation.

(1) *and* (2) The use of PMS and progesterone in the breeding of sheep out-of-season has already been commented on. Further references will be found in a recent review by Cowie & Folley (1955) in which work on superfoetation is also mentioned. Recently the results of an extensive experiment on the augmentation of fertility in Iceland ewes have been given by Pálsson (1956).

(3) There are now several fairly reliable methods for superovulating cows with gonadotrophic hormone preparations which have been considered in some detail in an excellent review by Willett (1953). A refractory condition develops if repeated attempts are made to superovulate the same

cow (Willett, Buckner & McShan, 1953) which is apparently not due to the presence of anti-substances in the blood of the cows so treated (Willett & McShan, 1955). It is possible to produce superovulation in calves before the end of the first month of post-natal life, but the number of ova produced is small and the percentage fertilized is low (Black, Ulberg, Christian & Casida, 1953; Marden, 1953).

(4) Recent experiments on the postponing of oestrus by injections of progesterone have already been noted. Injections of progesterone followed by PMS have been used in heifers (Nellor & Cole, 1956) and in ewes (Robinson, 1956) for this purpose. Further references to this topic will be found in reviews by Dutt (1953) and Ulberg (1955). Dowling (1954) recommends manual expression of the corpus luteum for synchronizing ovulation for purposes of insemination. No detrimental effects on the subsequent fertility rate have been observed after enucleation of the corpus luteum (Teige & Jakobsen, 1956).

Sperm transport

In 1952 VanDemark & Hays suggested that the uterine contractions evoked in the cow after mating facilitated the transport of sperm, and that the contractions were elicited by oxytocin released from the neurohypophysis. A critical survey of the experimental data led Cross (1955*c*) to conclude that the evidence for a neurohypophysial role in this process was suggestive but not conclusive. Further observations have since been published by VanDemark & Hays (1955). They noted that sperm transport occurred in the isolated perfused genital tract removed from a cow in oestrus and that the rate of transport was enhanced on adding oxytocin to the perfusate; on the other hand, no transport of sperm occurred in perfused tracts removed from cows in the progestational phase of the cycle until oxytocin was added to the perfusate.

Different zones of the myometrium of the bovine uterus vary quantitatively in their response to oxytocin under different physiological conditions, and the contracting patterns of reactivity seen at oestrus and at term may be correlated with the requirements of sperm transport and the expulsion of the foetus respectively (Fitzpatrick, 1957).

Studies on the effects of oxytocin and adrenaline on the metabolism of uterine muscle have been the subject of a preliminary report by Inchiosa & VanDemark (1956).

In view of the evidence for oxytocin release in the female during coitus, Cross (1955*c*) has made the interesting suggestion that a possible parallel may exist in the male. In preliminary studies he has observed that in the rabbit oxytocin appears to stimulate the contractile mechanism of the testis and epididymis, and it is suggested that sperm migration from the seminiferous tubules may be facilitated by such a process.

HORMONES AND INFERTILITY

Since the normal hormone patterns in the ruminant are only now being elucidated, it is scarcely surprising that no great advances have been made in the analysis of endocrine dysfunctions.

In 1949 Garm put forward the view that cystic ovarian degeneration in the cow was not a disease entity but part of the picture of two multi-glandular syndromes, namely nymphomania and adrenal virilism. In nymphomania, in addition to excess oestrogen production by the ovary, Garm deduced from histological and histochemical studies of the adrenal that there was an excess secretion of corticosteroids and of mineralocorticoids (aldosterone?) in particular. In adrenal virilism, on the other hand, he believed that there was an abnormal secretion of androgenic steroids by the adrenal cortex. Structural abnormalities have been described in the adrenals of nymphomaniac cows by Wayman & Asdell (1952), but since similar abnormalities were observed in the adrenals of old cows their significance is unknown. The occurrence of myeloid nodules and various types of degenerative changes in the adrenal cortices of infertile cows and bulls have also been described (Weber, McNutt & Morgan, 1950; Cupps, Laben & Mead, 1953, 1954, 1956). Further study is clearly required before the roles of the hormones of the adrenal cortex in infertility become clear.

An important study of the relationship between oestrogen and progesterone and the susceptibility of the uterus to infection has been made in the rabbit by Black, Simon, McNutt & Casida (1953) and in the cow by Rowson, Lamming & Fry (1953). The latter workers have shown that in ovariectomized cows exogenous progesterone promoted conditions in the uterus favourable to the growth of certain bacteria, whereas oestrogens rendered the uterus resistant to infection. These observations, of considerable clinical import, lend strong support to the view that some types of infertility arise through a combination and interaction of endocrine and bacterial factors (see also (38, 41, 196)).

To investigate the possibility that there might be a deficiency of luteotrophin in cows which repeatedly fail to hold to service, Kiddy, McShan & Casida (1956) assayed the prolactin in the pituitaries of ten 'repeat breeder' cows and ten first-service dairy cattle slaughtered on the 16th day of the cycle. No significant differences between the two groups were observed. Some success in treating repeat breeders has been claimed by Hill (1954), using injections of PMS during the pro-estrous phase of the cycle (16th and 17th days), and by Dawson (1954) who implanted progesterone pellets. Less decisive results as to the value of progesterone in such cases have, however, since been reported (Wiltbank, Hawk, Kidder, Black, Ulberg & Casida, 1956).

The treatment of cystic ovaries in dairy cows has been discussed by Roberts (1955), who, in a study extending over 10 years, obtained beneficial effects with sheep pituitary gonadotrophin and human chorionic gonadotrophin. He does not recommend manual rupture of the cysts.

Ovarian dysfunction (persistence of corpora lutea and appearance of ovarian cysts) has been observed in heifers maintained on a carotene-deficient diet indicating a disturbance of the hormone balance between pituitary and ovary (Jaśkowski, Walkowski, Dobrowolska & Domański, 1956). Other studies on endocrine aspects of nutritional-reproductive interactions have recently been discussed in an excellent review by Meites (1953).

A deficiency of progesterone prior to parturition has been suggested as the causal factor in some cases of retained placenta (McDonald, McNutt & Nichols, 1954).

In cows, injections of dienoestrol have been successfully used to dilate a constricted cervix which had previously prevented intra-uterine insemination being carried out (Munro, 1956).

The injection of stilboestrol 1–3 days before the next expected oestrus may be used to confirm that conception has occurred in cows, ewes and goats according to recent studies by Japanese workers. In non-pregnant animals oestrus is always induced within a few days of the injection, but no heat is induced if the animal is pregnant; there are said to be no harmful effects in either pregnant or non-pregnant animals (253, 254, 255, 256, 348).

The relationship of the thyroid to reproductive disorders has been investigated by Allcroft, Scarnell & Hignett (1954) who have obtained evidence of the existence of hypothyroidism in cattle in Great Britain; further studies are necessary to elucidate the significance of this finding.

Hormonal therapy (gonadotrophins and androgens) has given disappointing results in the treatment of infertility in bulls (Gassner, 1955). The effects of administering gonadotrophins and androgens to rams with poor libido have been studied by Ahmed (1955).

The existence of hormone deficiencies in inherited physiological defects of reproduction has been discussed recently by Rollinson (1955).

PINEAL GLAND

Santamarina & Venzke (1953) have reported on the weight changes of the pineal gland in cattle and sheep during various phases of the reproductive cycle. The gland was heavier in pregnant than in non-pregnant ewes; in the non-pregnant ewe large pineal glands were associated with the presence of well-developed ovarian follicles. Dairy cows had larger pineals than beef breeds. The pineal was heavier in the male, its weight being reduced after castration. These observations confirm earlier reports that some connexion appears to exist between the pineal gland and the reproductive processes, but the functions of the gland remain obscure. An extensive review of the literature on the pineal gland has recently been made by Kitay & Altschule (1954).

MAMMARY GROWTH AND LACTATION

Ovarian hormones

Investigations, particularly of a quantitative nature, into the role of oestrogen and progesterone in mammary development and lactation continue in laboratory animals, as well as in goats, cattle and sheep. The evaluation of the importance of oestrogen in the development of the mammary gland duct system in mice and rats has been objectively determined by the use of the technique of relative growth analysis first described by Huxley (1932). It has been shown by Flux (1954*a*) that normal growth of the duct system in ovariectomized mice can be obtained by giving 0·055 μg. oestrone daily from the 21st day of age, until the 42nd day following ovariectomy 14 days after birth. Silver (1953) made a similar investigation in the rat and obtained a normal type of mammary development in ovariectomized rats by giving oestradiol dipropionate in increasing doses 0·1 μg./2 days to 1·5 μg./2 days over a period of 60 days after weaning. Richardson, F. L. (1953, 1955) has also studied the relative growth of the duct system in mice.

The combined effects of progesterone and oestrogen in bringing about lobule-alveolar growth in the mouse and rat have been the subject of numerous papers, and there is general agreement that good lobule-alveolar growth is obtained with this combination in both sexes (42, 84, 109, 326, 384, 389, 390). Kirkham & Turner (1953, 1954*a*, *b*) have used the nucleic acid content of the rat mammary gland as an index of development in studies on the effects of ovarian hormones in rats under various conditions. This method has been used also in studies on the rabbit mammary gland (Yamamoto & Turner, 1956). The significance of dosages and ratios of the ovarian hormones concerned with mammary development in different species is discussed in a paper by Benson, Cowie, Cox & Goldzveig (1957), who investigated the roles of these hormones in mammary growth in the guinea-pig. Their findings indicate that in this species, contrary to previous beliefs, progesterone is necessary for optimal development of the mammary glands. For further references to work on laboratory animals see the fully documented reviews of Folley (1952, 1956).

Turning now to investigations into the role of the ovarian hormones in mammary development in ruminants, further work has been reported by the Shinfield group on the goat. In a paper by Benson, Cowie, Cox, Flux & Folley (1955) the results of further experiments are reported, which show that although oestrogen (hexoestrol) alone can bring about udder development and lactation, udders so developed exhibit various abnormalities and milk yields fluctuate widely. When progesterone is administered as well as hexoestrol the udders resulting from the treatment appear normal in structure and milk yields are much less variable. The optimal dosage for udder development, as described in this work, was 0·5 mg. hexoestrol and 70 mg. progesterone daily for 150 days. In this experiment final treatment with oestrogen (see Reineke, Meites, Cairy & Huffman, 1952) given with the object of initiating the secretory phase (triggering dose of oestrogen) failed to have any significant beneficial effect. As in earlier work quantitative estimations have been made of the total secretory area of the udders, using the method described by Richardson, K. C. (1953), together with two modifications. The correlation between the surface area so estimated and the milk yield obtained from the same half before removal and also from the contra-lateral half, left intact, was in all cases high. In an extension of these quantitative techniques the synthetic activity of a single alveolar secretory cell has been estimated (Richardson & Folley, 1954). Other studies on mammary development in the goat include that of Naito, Shoda & Nagai (1955), who attempted to relate the structural condition of the mammary gland at various stages of pregnancy and lactation to the functional capacity. Various characteristics such as the number of alveoli per lobule and the sectional area of the alveolus have been used as structural criteria to detect differences at the various stages of lactation. (See also a report by Naito, Yokoyama, Yoshioka, Yokoyama & Yokotsuka (1953).)

Lactation was induced in goatlings by thrice-weekly injections of 0·5 mg. diethylstilboestrol for 50 days by Eaton, Simmons, Sykes, Wrenn & Hall (1953). The lactational results were subsequently correlated with those from the first natural lactation, the correlation coefficients exceeding 0·6 based on three different criteria. All were highly significant. The authors suggest that

animals should be culled on a basis of response to this test. Goto & Oshima (1955), in a similar type of experiment, have induced lactation with stilboestrol in intact and ovariectomized goats. They gave a series of five injections which were repeated whenever lactation appeared to be falling. Maximum yield was obtained after two to four series of injections. They correlated such induced yields with later natural yields and obtained a coefficient ($r = 0{\cdot}783$) which agrees well with that of Eaton *et al.* quoted above.

In six goats in mid lactation single intramuscular injections of a synthetic oestrogen in crystalline form resulted in immediate declines in milk yield, lactation ceasing altogether by the 7th to the 11th day after treatment in four animals (Ajello & Composto, 1955). Desclin & Derivaux (1953), however, in a similar experiment, gave doses of diethylstilboestrol (0·25–20 mg.) to lactating goats for 5–35 days. The effects on milk yield seemed to depend on the initial daily milk yield which was depressed if it was greater than 300 ml. and increased if it was less than 200 ml. They suggested that when the initial yield was high, the effect of oestrogen was a direct one on the mammary gland, but that when it was low the oestrogen stimulated prolactin production by the pituitary. The use of crystal suspensions of oestrogen and progesterone has been investigated by Flux and Folley, who obtained good results by giving the hormones in this form (see Folley, 1956).

Other reports of successful lactations in goats following administration of oestrogens have been published (183, 332, 346).

In cattle, studies on mammary growth include a report by Wallace (1953) on an investigation of development at foetal stages and up to 6 months old. She found that development occurred at the same rate in both sexes until just before birth, but that from birth to 6 months of age mammary growth was dependent on ovarian oestrogens. She also investigated the same problem in lambs, and concluded that in them mammary development up to 4 months of age was not dependent on the ovaries. Yamamoto & Turner (1955) and Ruppert, Yamamoto & Turner (1956) describe udder growth obtained by administering 100 mg. progesterone and 100 μg. oestrogen daily to sterile heifers for 6 months. Lactation was then initiated with daily injections of 3·0 mg. oestrogen for 2 weeks. Two heifers reaching maximum production yielded 66 and 137% of the milk given by their half sisters, and another heifer yielded 106% of the production of its twin. Ruppert *et al.* also arrested or reversed the decline in lactation by feeding 10 mg. diethylstilboestrol daily for 4 weeks. Williams, Childs, Smith & Turner (1955) reported stimulation of mammary development in a manner very similar to the above, with lactation initiation brought about in the same way by giving oestrogen alone for 10 days. Hancock, Brumby & Turner (1954) also report on mammary development, this time in identical twin heifers. Dosages were employed similar to those used in the work mentioned above, 50–100 mg. progesterone being given together with 50–100 μg. diethylstilboestrol daily for 5 months, and again lactation was initiated by giving a high dosage (2–8 mg.) of diethylstilboestrol over the last 30 days. The responses obtained were compared with those of twin mates after normal pregnancy. Of the seven pairs of twins used, three sets were discarded because of low yields (both induced and normal) and the experimental animals of the four remaining sets produced 42–72% of the fat-corrected milk-yield of their mates.

In a brief report on the effects of oestrogen alone on the duct system, Reece (1955) failed to stimulate its development in fourteen sexually immature heifer calves by giving cutaneous and subcutaneous oestrogen although teat growth occurred. In combination with other hormones oestrogen appeared to stimulate development of the cistern region.

Other publications on the subject of ovarian hormones and lactation include those of Browning, Marion, Fountaine & Gier (1956) and Sykes, Wrenn, Kane & Jacobson (1956) dealing with the effects of feeding oestrogens to lactating cows, and those of Dziuk, Koshi & Petersen (1955) and Dalton (1955) who gave an alternating treatment of progesterone and oestrogen. Perez Matus & Ponce Pacheco (1952) report on the lactation induced by diethylstilboestrol alone, administered by a subcutaneous implant.

Sheep have also been subjected to oestrogen treatment, and Underwood, Shier & Petersen

(1953) described the effects of a prolonged series of stilboestrol injections on ewes which received 1–16 mg. daily intramuscularly for 6 months. Mammary enlargement was seen for 3 months, but was followed by regression, especially on the highest dose. McPherson (1955) described the induction of lactation in barren anoestrous ewes by stilboestrol either as tablets or in oily solution. A single injection of 40 mg. stilboestrol dipropionate was most effective and the ewes reared top quality lambs. This finding, however, was not confirmed by Lambourne (1956), who obtained only mediocre results in Romney ewes following a single injection of stilboestrol dipropionate in oily solution, and there was a wide variation between individuals. Strain differences may explain the divergence between the results of these two workers.

Oestrogens in milk. A few papers have dealt with the problem of the presence of oestrogens in milk after oestrogen administration. Szumowski (1951) fed rats on milk from a heifer with a stilboestrol-induced lactation and observed changes in the reproductive organs of female rats, indicative of the presence of small amounts of oestrogen in the milk. A brief report by Turner (1956) on the oestrogen contents of normal milk and the results of feeding synthetic oestrogen has also appeared. He found a small amount of oestrogen in commercial milk, and variable and increased quantities in milk from pregnant cows. Non-pregnant cows appeared to have little or none, and the feeding of 10 mg. of diethylstilboestrol to non-pregnant cows appeared to be without effect.

The effect of oestrogens on milk composition. Folley (1936) and Folley, Scott Watson & Bottomley (1941) first showed that the administration of oestrogens to lactating cows could induce substantial increases in the percentage total solids in milk. Although the rise in total-solids percentage was sometimes accompanied by a significant decline in milk yield, in general the milk yield remained unaffected. In a series of experiments conducted at the N.I.R.D., Shinfield, Mr J. B. Hutton (personal communication) has confirmed these earlier findings, and has shown that by use of appropriate doses of oestrogen significant increases of total solids can be obtained without depressing the milk yield.

Oestrogens in pasture. The existence of oestrogens in pasture plants and their effects on reproduction and milk quality has been the subject of further investigation in both this country and Australia. The subject, however, has been comprehensively reviewed in recent years (18, 50, 265), and the reader is referred to these excellent papers.

Relaxin

The possible role of this hormone in mammary development has been studied in several species. In the rat, rabbit and guinea-pig it appears to enhance the effect of the main ovarian hormones (Garrett & Talmage, 1952; Smith, 1954). In the sheep it has been reported to exhibit a milk-ejection effect, and the suggestion is made that this is obtained by the same mechanism as the oxytocic action, i.e. contraction of the alveoli (Shaffhausen, Jordan & Dracy, 1954). Experiments with goats at Shinfield (Benson, Cowie, Cox & Folley, unpublished) have indicated that relaxin, when given together with oestrogen and progesterone to induce udder development, has an inhibiting effect on the lactation.

Anterior-pituitary hormones

The importance of the hormones of the anterior pituitary in mammary development and lactation has been recognized for many years, though there has been some dissension as to the possible manner in which the various hormones are related to these two phenomena. As is well known, Dr C. W. Turner and his colleagues many years ago postulated the production by this gland of specific mammogenic hormones distinct from the other hormones that it was known to produce (for review see Folley, 1952). More recently, however, evidence has accumulated which would seem to render the postulated existence of these mammogens unnecessary.

In the rat and mouse Lyons and his collaborators have studied the roles of various anterior-pituitary hormones in mammary development and lactation and their results are reported in a number of papers (84, 203, 204, 205, 206, 207). They have demonstrated in the rat that in the absence of

the pituitary gland, adrenals and ovaries, a regimen of oestrone, progesterone, prolactin and GH was most efficacious in bringing about lobule-alveolar development which was less well marked if GH was omitted. Their treatment was given for a period of 1 week, and subsequently milk secretion could be initiated by giving further treatment with prolactin, GH, thyroxine and cortisone. Successful lobule-alveolar development has also been obtained in hypophysectomized-gonadectomized male rats, both by injection of the hormonal tetrad of oestrogen, progesterone, prolactin and GH, and by their local application in the form of compound pellets adjacent to the glands. Some duct development was also obtained in such male rats with oestrogen and GH with or without progesterone.

Further to these experiments, the work of Ray, Averill, Lyons & Johnson (1955) is of interest. They demonstrated that extracts of 12-day-old placentae of rats had prolactin-like activities as judged by various criteria including the pigeon crop response, and these extracts could replace prolactin in the development of mammary glands in hypophysectomized-ovariectomized rats. Ferrara (1952*a*, *b*) reports increased and prolonged lactation in ewes following the administration of placental extracts. It would appear that, in late pregnancy at least, the placenta is capable of producing or storing either prolactin or a substance having similar properties.

Mizuno, Iida & Naito (1955) have investigated the role of prolactin in mammary alveolus formation in the rabbit using the technique of intraduct injection, and concluded that prolactin can stimulate both duct and alveolus formation according to the stage at which the intraduct injections were given.

Prolactin has, of course, for some time, been considered to be of principal importance in lactogenesis and its significance in the initiation of lactation has been dealt with by Meites and his co-workers in a series of papers (220, 221, 222, 309). These workers find that if oestrogen and progesterone are given in the amount necessary for producing mammary growth in spayed rabbits, milk secretion as a result of prolactin administration is inhibited unless 4 mg. or more of prolactin (20–25 i.u. per mg.) is given. This compares with the initiation of lactation obtained with 2 mg. of prolactin (20–25 i.u. per mg.) when sub-optimal amounts of oestrogen and progesterone were given. Oestrogen or progesterone alone, when given with prolactin, caused at the most only a slight inhibition of the secretory response. The authors conclude that at the dose-levels employed the two ovarian hormones together can inhibit the lactogenic action of prolactin. This work is of particular interest in relation to the debatable question of lactation initiation following pregnancy. These findings seem to be at variance with the well-known Meites-Turner theory of lactation initiation as originally postulated, though later modified by Meites (1954). It is a question critically discussed by Folley (1956), who advances a tentative theory aimed at reconciling the rather conflicting evidence; the reader is referred to Dr Folley's book for details. Other work on the initiation of milk secretion has been published by Bruley, Simmonnet & Tisseur (1953).

The manner in which prolactin brings about secretion has been investigated by Williams & Turner (1954, 1955) who have shown, in the rabbit, by using prolactin labelled with ^{131}I, that the prolactin becomes associated with the nucleoproteins of the cytoplasm, and not with the lipids assuming no splitting off of the ^{131}I occurs. This is taken to indicate that prolactin brings about changes in enzyme activity which then alter the synthetic activity of the cell.

The release of prolactin from the anterior pituitary is a subject which has recently received some attention, but this will be referred to in the section dealing with the posterior pituitary.

The galactopoietic effect of anterior-pituitary extracts in cows is well known, and in recent years the particular importance of GH in this respect has been clearly demonstrated and has been reviewed by Folley (1955). Various groups of workers have shown that when growth hormone is administered to dairy cattle milk and butterfat yields are both considerably increased (for reference see Folley, 1955, also Shaw, Chung & Bunding, 1955).

Chung (1955) obtained large increases in milk yield by administering GH intramuscularly to one of each of two pairs of identical twin heifers for about 24 days before its first calving. Given *post-partum* to older cows, GH maintained an increased production for 7 weeks, with a

fall to normal on discontinuing the injections (see also Shaw, 1955). In a similar experiment, however, Brumby (1956) was unable to confirm these findings. Meites (1955) reports definite increases in milk production with GH in goats, but found that in rabbits GH greatly depressed milk secretion when given together with prolactin, the latter hormone having markedly stimulated secretion when administered alone (see also Meites & King, 1954). The possible implication of the diabetogenic action of GH is here discussed in relation to these findings. A surprising finding, contrary to the majority of evidence which ascribes a galactopoietic activity to GH, is that of Flux (quoted by Folley, 1955) who obtained no galactopoietic effect in cows or goats with GH administration. As pointed out by Folley, it seems likely that the animals in these experiments were either in some way refractory to the galactopoietic actions of GH, or that the diabetogenic action of GH may have been involved. Wrenn & Sykes (1952, 1953) demonstrated that the administration of GH and TSH had a greater galactopoietic effect than TSH alone, which in turn was more effective than prolactin, in young heifers with udders developed by oestrogen-progesterone treatment. In sheep, Jordan & Shaffhausen (1954) have obtained highly significant increases of milk yield by administering 25 mg of GH preparation daily, and Dracy & Jordan (1954) compared the effectiveness of the same dosage of bovine and porcine GH preparations over 5 days in the ewe. They found that the bovine extract was the more active and had a longer lasting effect.

Mr J. B. Hutton (personal communication), using a single injection technique on identical twin cows, has shown that the extent of the increase in milk yield following injection of GH, is linearly related to $\log_{10}$ of the weight of the growth hormone injected. By measuring the responses in milk yield for 4 days after each injection, a maximum increase of 12% has been calculated. Alternatively, if the galactopoietic capacity of the GH is estimated from the 24 hr. milk yield during which the maximum response to each injection is obtained, then the increase is 20%.

Referring back to the work of Sykes and Wrenn with TSH, several workers, including Chung (1955), Brumpy & Hancock (1955) and Shaw (1955), have obtained little or no galactopoietic effect with this hormone.

Some investigations have also been made into the effects of ACTH on milk yield, and a depression in yield following treatment with this hormone has been observed in cattle by Flux, Folley & Rowland (1954) and Shaw, Chung & Bunding (1955). Its effects on mammary development in laboratory animals are somewhat conflicting, being inhibitory to oestrone induced mammary development in spayed mice (Flux, 1954*b*) and stimulatory (though less so than cortisone and cortisol) to duct and lobule-alveolar development in normal female rats (Johnson & Meites, 1955*b*). A mammotrophic and lactogenic effect of ACTH was reported for the rat by Selye (1954*a*).

Adrenocortical hormones

As mentioned under the section on the anterior pituitary, it seems on the available evidence that ACTH has the effect of decreasing rather than increasing milk yields, but it is nevertheless clear from past work on the effects of adrenalectomy that the adrenals are necessary for the successful maintenance of lactation. Deoxycorticosterone acetate (DCA) and cortisone have been shown to provide the best replacement therapy in the lactacting rat after adrenalectomy, and it has also been recently shown that an ovarian secretion, probably progesterone, contributes significantly to the partial lactation maintenance observed after adrenalectomy (see Folley, 1956). Aldosterone and cortisol and its 9-α halo derivatives have also been shown to be effective in maintaining lactation following adrenalectomy in the rat (Cowie & Tindal, 1955). These authors have also recently studied the maintenance of lactation in the goat after adrenalectomy (Cowie & Tindal, unpublished work). Five lactating goats were adrenalectomized and implanted subcutaneously with tablets of DCA and cortisone acetate, giving an average absorption of 2–4 mg. DCA and 7–12 mg. cortisone acetate per day. Lactation was maintained in all five goats, although in four the yield was somewhat reduced. After some months the DCA tablets were removed from three of the animals, and there was a precipitous drop in milk yield and the goats died within 4 days, although they were still implanted with cortisone acetate. The cortisone

acetate tablets were removed first from the other two animals and there was a gradual fall in milk yield; one of these goats died 3 weeks later while still implanted with DCA, the other living until the DCA tablet was removed. In the ruminant, as in the rat, both cortisone and DCA appear to be necessary for the maintenance of normal lactation. The effects of adrenal steroids on mammary gland growth have been studied only in mice and rats (114, 178, 179, 306, 307, 308). Reference to the effect of cortisone on the initiation of lactation, when administered together with prolactin and/or GH to spayed female rabbits following oestrogen and progesterone treatment, is made by Meites & King (1954). In these circumstances cortisone appeared to have an inhibitory effect on lactation initiation. However, Meites (1955) reports no depression of lactation in the goat following cortisone administration.

Thyroxine and thyroid-active substances

Investigations into the possible galactopoietic action of thyroxine and iodoproteins continue to appear, and in general the results bear out earlier work which, as reviewed by Smith (1953), indicated that the galactopoietic effects obtained are temporary in nature and often correlated with a reduced length of lactation. Nevertheless, much work has been done recently on this problem. The synthesis in 1949, by Chalmers, Dickson, Elks & Hems, of L-thyroxine made economically possible the use of the hormone in quantity, and this synthetic product was shown by Bailey, Bartlett & Folley (1949) to be potent when administered orally to cows. It has several advantages over iodocasein, being odourless and tasteless, and its purity is assessable by the use of chemical procedures, so no bioassay is necessary as it is with iodoprotein. Its use also obviates the large iodine intake which the use of iodoprotein entails.

Regarding the possible deleterious effects of the administration of thyroid-active substances, Leech & Bailey (1953) have made an extensive survey of the affects on health of cows treated with L-thyroxine and iodocasein. They found no deleterious effects over a 3-year period from either of these thyroid-active materials, and observed that although an increase in milk yield could be obtained, it was often largely negatived by shortening of the lactation period. There was no significant difference in response to the two preparations, but cows ate food containing thyroxine more readily than they ate the iodocasein diet. A similar study was made by Aitken, Boyne & Crichton (1953).

Hancock, Brumby & Turner (1955) administered L-thyroxine to six identical twin heifers during the first 6 months of pregnancy, and in comparison with their untreated mates these heifers showed a depressed rate of gain of body condition, and the subsequent milk and butterfat production of most of them was also depressed.

Naish (1954) has described beneficial effects on lactation of thyroid administration in the human.

The milk composition in three breeds of cows as affected by thyroxine has been investigated by Rossi (1953); butterfat, whey protein, lactose and total solids increased while casein, total N and ash decreased. Chanda (1956) has studied the effect of L-thyroxine on carotene and vitamin A metabolism in cows, and found that oral dosing caused greater concentration of vitamin A ester in blood serum and milk than parenteral administration. The converse was true for β-carotene.

A substance said to be 5–7 times more active than L-thyroxine in various biological tests in small animals and humans was isolated recently (for review see Folley 1956) and has been identified as 3:5:3-triiodo-L-thyronine. When tested for galactopoietic activity by Bartlett, Burt, Folley & Rowland (1954), it was found to have very little activity when administered orally, much less than L-thyroxine, but by subcutaneous injection it proved to be somewhat more active than the latter substance. They concluded that the low oral potency, probably due to a more rapid inactivation in the rumen, rendered it unsuitable as a galactopoietic agent.

Studies with iodinated casein in cows have continued to appear (126, 128, 152, 153, 154, 321, 334, 343, 344), and in general indicate a temporarily enhanced milk yield as a result of the treatment. Reports on other species include those of Weits, Houtman & Roborgh (1953) in goats, Müller &

Ženišek (1955) in sows, and Giraud & Coignet (1953) in the human. All report increases of milk yield following treatment.

The way in which the thyroidal effects of iodocasein are mediated is discussed in a paper by Friedberg & Reineke (1956), in which the metabolism of ^{131}I labelled iodocasein is traced in the rat. They conclude that the thyroxine content of iodoprotein is released in the body, principally by the liver, and the thyroidal action is then directly attributable to this free thyroxine. The thyroxine content of iodinated proteins has been shown by Reineke (1954) to be approximately 1·0%. Other studies concerning the metabolism of thyroid-active substances have been concerned with their secretion in the milk. Lengemann, Monroe & Swanson (1955) have shown that 5% of a dose of ^{131}I given intravenously or orally to the cow appears in the milk within 7 days, the rate of secretion being unrelated to milk yield or dosage. The first detectable quantity of ^{131}I in the milk appeared within 30 min. of administration, indicating rapid absorption from the rumen. In the dog, Middlesworth & Hansey (1954) observed a rapid excretion of ^{131}I in the milk, 5·9% appearing in 6 hr. after injection, and a further 5% by 12 hr. after administration. Between 30 and 60% of this ^{131}I was protein bound, a figure which was greatly reduced by the administration of thiouracil. In another paper, Middlesworth (1956) has studied the effect of casein on iodide metabolism. He found that a high casein diet appears to inhibit thyroid function, and suggests that digestion of casein may release a thyroid inhibitory substance. Wright, Christian & Andrews (1955) observed in the goat that when ^{131}I was administered as iodocasein, 15·5% was transferred to the milk in 7 days, but when the ^{131}I was administered as iodide, 50% was transferred.

The secretion of administered ^{131}I in the milk has been demonstrated in the human by Nurnberger & Lipscombe (1952) and by Miller & Weetch (1955), the dangers to the infant being pointed out by the latter authors. In this connexion Rugh (1953) has discussed the sterilizing effect of ^{131}I transmitted via milk on young mice. He found that the ovary is much more sensitive to the ionizing radiations of radioiodide than are the testes.

Brown-Grant (1956*a*) has demonstrated ^{131}I excretion in the milk by the rabbit, and has discussed its implications in relation to thyroid activity. He concludes that the increased iodine excretion is the cause of the apparent changes in thyroid activity which occur during lactation, and that they are not due to alteration in the pituitary-thyroid relationships. He has also demonstrated (Brown-Grant, 1956*b*) that the iodide concentrating mechanism of the mammary gland is similar to that of the thyroid and salivary gland in many respects. This iodide concentrating ability of mammary tissue appears to be possessed also by the bovine, according to the figures published by Binnerts (1954), though Glascock (1954) found no apparent concentration of ^{131}I in the milk from a Shorthorn cow following the administration of labelled iodide.

Turning to another aspect of interest, that of the secretion rate of the thyroid gland itself, one of the indicators of this activity is the plasma level of protein bound iodide (PBI). This has been shown to be related to the thyroid secretion rate in many species, and Lewis & Ralston (1953) have endeavoured to assess the normal range in cattle together with various environmental effects. The factors affecting the variations found have been examined, and amongst other findings these authors have shown that the PBI concentration decreased with age, and that it varied considerably within and between animals. Sørensen (1956*a*) has made a similar survey and confirmed these findings. He also reports a positive correlation between butterfat yield and PBI concentration in the plasma of lactating cows.

In other studies on normal thyroid activity in dairy cattle, the thyroid uptake of the tracer element ^{131}I has been used. Pipes (1956) assessed thyroid activity by three different methods and found a significant correlation between thyroid activity and peak milk production at a subsequent lactation.

Blincoe (1953) has published an *in vivo* technique for determining ^{131}I uptake by the thyroid of the cow, by using a Geiger-Müller counter in contact with the neck-skin over the regions from which maximal count rates were obtained, these corresponding to each lobe of the thyroid gland. Counts so obtained were compared with those obtained from a known amount of ^{131}I in a model

of a cow's neck and calculated as a percentage of the injected dose (see also Blincoe & Brody, 1955*a*, *b*).

This method and others have been used by Sørensen (1956*b*) to determine thyroid secretion rate in the cow. This worker demonstrated that there is a positive correlation between daily butterfat production and thyroid secretion rate, and his paper includes a useful review of work on iodine metabolism and thyroid function. A similar conclusion regarding the relationship between thyroid activity and lactation was reached by Singh, Henneman & Reineke (1954, 1956) for the ewe. Henneman, Reineke & Griffin (1955) have described their technique for measuring the daily thyroid secretion rate in the sheep; like Blincoe's technique it involves the injection of ^{131}I and a count in the neck region using a Geiger-Müller counter. These authors investigated the relationship between output and pregnancy, lactation, and seasonal and age differences. They confirmed again that lactating ewes have a higher thyroid output than non-lactating ewes, whereas pregnancy does not cause any significant differences in thyroid secretion.

In laboratory animals experiments have yielded somewhat conflicting results when considered with reference to the necessity or otherwise of the thyroid in mammary growth and lactation. Species differences may well exist in regard to the exact role played by the thyroid in these processes. For example, Casa (1954) found that thiouracil inhibited lobule-alveolar development in response to exogenous oestrogens in spayed mice, and Cohen (1953) showed that thiouracil given to pregnant guinea-pigs had no effect on pregnancy but brought about a failure of lactation 1–3 days after its normal onset. Withdrawal of thiouracil allowed lactation to recover. However, Chen, Johnson, Lyons, Li & Cole (1955), using hypophysectomized, ovariectomized, adrenalectomized rats from which the thyroids were also eliminated either surgically or by ^{131}I treatment or both, obtained extensive mammary development and a copious lactation by treatment with oestrogen, progesterone, GH, cortisol and prolactin, without the addition of thyroxine. Lactation did not appear to be impaired by a 50% reduction in metabolic rate.

Pitney & Fraser (1953) have investigated the mode of action of antithyroid drugs and conclude that thiouracil inhibits the iodinating enzymes, whereas another class of drug with antithyroid properties (e.g. resorcinol) removes iodine from combination with protein.

Swett, Mathews & Fohrman (1955) have studied the correlation between the weight of the thyroid and milk yield of dairy cows from different geographical areas in the U.S.A. In certain instances highly significant correlations were found, but results were so inconsistent that it would appear that in general there is no correlation.

Parathyroid hormone

Little work has been done on the role of the parathyroids in lactation, but it was demonstrated some years ago that lactation in the rat is severely impaired as a result of parathyroidectomy (Cowie & Folley, 1945), and these effects were thought to be due not to any direct effect of the parathyroid hormone in the mammary gland, but to the general impairment of calcium and phosphorus metabolism caused by the removal of these glands (Folley, 1952). Recently, however, some interesting results have appeared which indicate that the parathyroids may have a direct effect on the mammary gland, influencing the calcium-concentrating mechanism as well as the over-all metabolism. Thus, the calcium content of milk from parathyroidectomized rats showed no significant reduction from control levels, though as would be expected, the blood serum calcium level was much reduced (Munson, 1955). Other related work is that of Borgman & Rumbaugh (1956), who showed that dihydrotachysterol aided in the maintenance of lactation in thyroparathyroidectomized rats given iodinated casein.

Hormones of the neurohypophysis

The implication of a neurohormonal arc in the milk-ejection reflex has been further substantiated since the last review in this series (Smith, 1953). There can now be little doubt that the release of oxytocin from the posterior lobe of the pituitary takes place as a result of a nervous excitation following the milking or suckling stimulus.

Further evidence of oxytocin release following stimulation of certain regions of the hypothalamus has been presented by Andersson & McCann (1955), which confirms the several reports discussed by Smith in his earlier review. Eayrs & Baddeley (1956) have investigated the afferent pathways between teat and hypothalamus by studying the effect of sectioning the dorsal nerve roots and spinal tracts in the rat. They deduced from their experiments that the pathway by which the suckling stimulus maintains lactation enters the central nervous system via the dorsal roots and is conveyed via the spinal cord by pathways deep in the lateral funiculus of the same side. This problem has also been investigated in the goat and ewe by Denamur & Martinet (1954), whose results with their goats indicate that the dorsal roots are implicated in the transmission of this stimulus. They also showed that bilateral lumbar sympathectomy considerably reduced the milk yield compared with that of sham-operated controls. In the ewe, however, no significant differences were observed between operated animals and sham-operated controls in respect of milk yields.

The view that oxytocin causes contraction of the myoepithelial cells surrounding the alveoli, strong evidence for which has been accumulating over the past few years, has now been further substantiated by Linzell (1955), who has observed alveolar contraction after the direct application of oxytocin and vasopressin to the exposed mammary gland of the mouse, vasopressin activity in this phenomenon being about 8% of that of oxytocin. Silver (1954) has studied the distribution of alkaline phosphatase in the mammary glands of various species, and found the myoepithelium to possess marked activity. He has also (Silver, 1956) studied the changes occurring in the mammary gland following engorgement after nursing has ceased. He brought about mammary engorgement by either removing the litter, or by ligating the galactophores subcutaneously. After 24 hr. the congested glands responded to intravenous oxytocin injection (10 mμ.) by milk ejection. The histological appearance at this time was that of maximal alveolar distension, with patent capillaries present. However, between 36 and 48 hr. after commencement of congestion, there was a dramatic reduction in the patent capillary network, and the glands usually failed to respond to intravenous oxytocin (10 mμ.) though they always responded to direct application of 0·001 mμ. oxytocin. Attempts to re-establish lactation in non-suckled glands were successful only if the period in which the rats remained unsuckled was less than 110 hr. and biopsies from animals which did recover secretory activity revealed a refilling of the capillary network.

Great interest has been taken recently in the site of formation of oxytocin and other posterior-lobe hormones (for review see Harris, 1955), and evidence is accumulating which confirms the view that these hormones are produced, not by the posterior lobe but by certain regions of the hypothalamus. The use of the Gomori chrome alum haematoxylin stain (Gomori, 1939) and of other techniques (Adams & Sloper, 1956) which appear on the basis of strong evidence to be specific for demonstrating the neural lobe hormones, indicate that these hormones are produced in the cells of the supraoptic and paraventricular nuclei of the hypothalamus and pass along the axons of the hypothalamo-hypophysial tracts via the pituitary stalk to the posterior lobe, which serves to store them (for review see Scharrer & Scharrer, 1954). Olivecrona (1954) has produced evidence which implicates the paraventricular nuclei in particular with oxytocin production.

It has been shown by Stutinsky (1952) and by Billenstein & Leveque (1955) that after removal of the neural lobe in the rat the proximal end of the stalk becomes reorganized and is apparently capable of storing the neural lobe hormones which continue to pass down the stalk. This has been confirmed by Benson & Cowie (1956) who reported that following posterior lobectomy during the first lactation, rats were unable to nurse their young unless they received injections of oxytocin. Subsequently, however, the rats underwent a second pregnancy, littering quite normally and nursing their young without difficulty. Histological examination of the pituitary area showed that the cut end of the stalk had become enlarged. These findings may well explain the hitherto contradictory effects on lactation which have been obtained following removal of the neural lobe (for discussion see Benson & Cowie, 1956; Cowie & Folley, 1957).

The investigations of Whittlestone into the mechanics of the milk-ejection process have been

continued, and he has published a series of papers dealing with the intramammary pressure changes brought about in the lactating sow by oxytocin, vasopressin and some pharmacological agents (Whittlestone, 1945*a*,*b*,*c*). He found that 0·05 i.u. oxytocin was the minimum amount which would consistently produce a rise in intramammary pressure, but that if the injection was made slowly a higher dosage was necessary. Vasopressin possessed about 20% of the milk-ejecting activity of oxytocin, a figure which has been obtained previously by several workers (see Folley, 1956); acetylcholine had a similar effect to oxytocin, a finding confirmed by Linzell (1955) who showed that various pharmacological agents possessing smooth muscle stimulating properties had this effect when applied directly to the mouse mammary gland. Whittlestone (1955) has studied also the intramammary pressure changes during the milking process in the cow, and has discussed differences between cows and sows in respect of milk-ejection.

Studies on the relative potencies of the oxytocic and vasopressin octapeptides have appeared. It is apparent that vasopressin possesses an intrinsic milk-ejecting activity, and that its oxytocic effects are not all due to contamination by the oxytocic hormone. Oxytocin also possesses a slight antidiuretic action (80, 101, 338).

Much work on the effects on milk yield of the administration of oxytocin to lactating animals has been published in the past few years. The beneficial effect of oxytocin on yields has usually been ascribed to a reduction in intra-alveolar pressure, which in turn enables the secretory cells to function more efficiently. Recent experiments, however, indicate that increasing alveolar pressure does not significantly affect milk secretion rate until about 16–20 hr. after milking (Turner, 1955; Elliott & Brumby, 1955). Thus it may be that the effects obtained on milk yield and milk quality are due to other effects of the oxytocin, which will be discussed later.

Investigations into the effect of injections of oxytocin on the total milk yield and fat production in cattle have been made by Sprain and his colleagues (Sprain, Fosgate, Smith & Tyler, 1952; Sprain, Smith, Tyler & Fosgate, 1954), who obtained increases in both yield and butterfat content by administering oxytocin over alternate 14-day periods during lactation. Donker, Petersen, Harvey & Koshi (1952) have studied the effects of oxytocin on the lactation yield of one of a pair of very low-yielding monozygous twin heifers. By giving oxytocin after each milking, and then milking for a second time the yield of the heifer so treated was brought up to a normal level during its second lactation. These results are again reported in greater detail by this group (Donker, Koshi & Petersen, 1954*a*) and they show that about half the total milk produced was obtained during the second milking and that the butterfat content of this milk was 5·74% compared to 1·44% for the 'regular' milk. They also express the view that the stimulating effect of oxytocin cannot be solely accounted for by the extra space made available by the complete emptying of the udder obtained by oxytocin.

An interesting result of repeated administration of oxytocin was obtained by this group who milked a cow at hourly intervals with the aid of 20 i.u. oxytocin for a total period of 156 hr. This treatment caused disturbances in the normal milk-ejection reflex which took 7 days to subside. It is suggested by the authors that either a suppression of output of oxytocin by the posterior pituitary was brought about by the large amounts of exogenous oxytocin, or that the sensitivity of the myoepithelium to physiological amounts of endogenous hormone was lost (Donker, Koshi & Petersen, 1954*b*). Several other papers dealing with the effects of oxytocin on milk yield and quality by this group of workers have appeared (92, 95, 193).

Johansson and co-workers have published findings on the variations in fat percentage during normal milkings. They observed a rise throughout the process. It is believed that the fat globules are held up either in the alveoli or in the small ducts, and that the size of the globule affects the length of delay incurred in its ejection. Oxytocin injections following normal milking stimulated the let down of residual milk with a high butterfat content (Johansson, Korkman & Nelson, 1952; Johansson 1952).

Denamur (1953) has investigated the effect on milk secretion in the goat of giving repeated doses of oxytocin, and he considerably increased his total yields of both milk and butterfat. The same worker has also investigated the sensitivity of the goat mammary gland to posterior-

pituitary hormone (Denamur & Martinet, 1953) and has found that maximal responses were obtained with intravenous doses of 10 mu. but good responses were obtained with as little as 1 mu. Bílek & Janovsky (1956) have also studied the dose-response relationship between oxytocin administration and milk ejection. They injected doses ranging from 0·05 to 2·0 i.u. and found a quantitative relationship between dosage and amount of milk ejected, the latter rising as dosage was increased. Studies in human subjects by Haeger & Jacobson (1953) and Isbister (1954) have confirmed the efficacy of oxytocin in overcoming engorgement and nursing difficulties.

Very considerable interest has been taken recently in the effect of the sympathetico-adrenal system on milk ejection, and papers dealing with the inhibitory effect of adrenaline have appeared (51, 155, 192, 355, 370). In a series of papers on this subject Cross (1953, 1955*a*, *b*), working with rabbits, has elucidated the mechanisms concerned, and it seems clear that the secretion or administration of adrenaline has a peripheral effect on milk ejection in that it causes a vasoconstriction of the mammary blood vessels and thus prevents the circulating oxytocin from effectively stimulating the myoepithelium.

The fact that direct mechanical stimulation of the mammary gland (shown by Cross, 1954, to be effective in causing alveolar contraction) is not prevented by adrenaline, together with the work of Linzell (1955) previously discussed, on the effect of the direct application of oxytocin to the mammary gland, renders it very unlikely that adrenaline exerts any direct effect on the myoepithelium. This has been confirmed recently in Japan by Yokoyama (1956) who has repeated the experiments of Cross. However, this peripheral blocking effect, mediated by adrenaline, has been shown by Cross (1955*b*) to play only a minor part in the emotional inhibition of milk ejection, which is caused by a central block of the reflex arc, preventing the release of oxytocin from the posterior lobe. Cross (1955*c*) has himself reviewed these aspects of the milk-ejection reflex.

A new role for oxytocin has recently been postulated by Benson & Folley (1956, 1957). This ascribes to oxytocin the function of stimulating the release of prolactin from the anterior pituitary, a theory which fits in well with the known facts concerning prolactin release. It was suggested many years ago by Selye (1934) that suckling stimulated the release of prolactin and thus ensured the maintenance of the mammary glands as long as suckling was continued. This theory was substantiated by Hooker & Williams (1941) and others who showed that the mammary glands of lactating rats could be maintained by prolactin injections after removal of the pups. Benson & Folley have demonstrated the same effect of mammary gland maintenance by oxytocin injections and appear to have eliminated the possibility of a direct effect of oxytocin on the mammary gland, or the involvement of any impurity in the hormones administered. If this theory is correct, it would explain the findings of Donker *et al.* (1954*a*), and others mentioned above, who considered that the increased milk yields obtained by the use of oxytocin were more than could be accounted for by the reduction of intra-alveolar pressure by removing the residual milk. If oxytocin is indeed the stimulator for prolactin release, it fits in well with similar concepts for which evidence has been accumulating concerning the release of ACTH by the neural lobe hormones (for review see Fortier, 1956). This idea of the control by oxytocin of prolactin release has been tentatively postulated by a number of workers over the past years (56, 262, 366).

In concluding this section mention must be made of the successful synthesis of oxytocin by du Vigneaud and his collaborators (du Vigneaud, Ressler, Swan, Roberts, Katsoyannis & Gordon, 1953; Nickerson, Bonsnes, Douglas, Condliffe and du Vigneaud, 1954; du Vigneaud 1956) who have also assayed its milk-ejecting activity. A further synthesis of oxytocin has since been announced by Boissonas, Guttman, Jaquenoud and Waller (1955), the potency of which has been tested by Bainbridge, Nixon, Schild & Smyth (1956) and by Francis & Francis (1956).

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